IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	Buchter-Larsen et al.
Filed	:	November 5, 1999
Serial No.	:	09/423,126
For	:	A PROCESS FOR PREPARING AN ANTI-OXIDANT
Art Unit	:	1638
Examiner	:	Russell Kallis

745 Fifth Avenue, New York, NY 10151

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: **Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450**, on **May 24, 2004**.

Anne-Marie C. Yvon, Reg. No. 52,390
(Name of Applicant, Assignee or Registered Representative)

Anne-Marie C. Yvon
Signature

May 24, 2004
Date of Signature

COMMUNICATION

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Enclosed is a certified copy of priority documents for the above named application. Applicants hereby claim priority under 35 U.S.C. §§119 and 120 from International Patent Application No. PCT/IB98/00708 and United Kingdom Patent Application No. GB 9709161.5.

Acknowledgment of the claim of priority and of the receipt of said certified copy is respectfully requested.

Respectfully submitted,
FROMMER LAWRENCE & HAUG LLP

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Reg. No. 32,147

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PATENT COOPERATION TREATY (PCT)
TRAITÉ DE COOPÉRATION EN MATIÈRE DE BREVETS (PCT)

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International Application No. / Demande internationale n° PCT/IB 98 / 00708

International Filing Date / Date du dépôt international 06 MAI 1998

06. 05. 98

Geneva/Genève, 28 APRIL 2004

(28.04.04)

International Bureau of the World Intellectual Property Organization (WIPO)

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Head, PCT Receiving Office Section
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REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

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PCT / IB 98 / 00708
International Application No.

06 MAY 1998 (06.05.98)
International Filing Date

INTERNATIONAL BUREAU OF WIPO
PCT International Application
Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* PCT 500 1 CTH

RO

Box No. I TITLE OF INVENTION

A PROCESS OF PREPARING AN ANTI-OXIDANT

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

Danisco A/S
Langebrogade 1
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DK-1001 Copenhagen K
Denmark

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(i.e. country)* of nationality: Denmark

State *(i.e. country)* of residence: Denmark

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

BUCHTER-LARSEN, Aksel
Amager Felledves 27
DK-2300 Copenhagen
Denmark

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: Denmark

State *(i.e. country)* of residence: Denmark

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

HARDING, Charles Thomas
D Young & Co
21 New Fetter Lane
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United Kingdom

Telephone No. +44 1703 634816

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Teleprinter No. 477667 YOUNGS G

☐ Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

See Notes to the request form

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

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Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

MARCUSSEN, Ian
Knabröstrede 25
DK-1210 Copenhagen
Denmark

This person is:
- [ ] applicant only
- [x] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: Denmark

State *(i.e. country)* of residence: Denmark

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [x] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

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This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

[ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

☒ AP ARIPO Patent: GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT

☒ EA Eurasian Patent: AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☒ EP European Patent: AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☒ OA OAPI Patent: BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

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☒ AL Albania .....
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In addition to the designations made above, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except the designation(s) of _____.

The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

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Supplemental Box *If the Supplemental Box is not used, this sheet need not be included in the request.*

Use this box in the following cases:

1. If, in any of the Boxes, the space is insufficient to furnish all the information:	*in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient;*
in particular:	
(i) if more than two persons are involved as applicants and/or inventors and no "continuation sheet" is available:	*in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below;*
(ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked:	*in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;*
(iii) if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America:	*in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;*
(iv) if, in addition to the agent(s) indicated in Box No. IV, there are further agents:	*in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;*
(v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "patent of addition," or "certificate of addition," or if, in Box No. V, the name of the United States of America is accompanied by an indication "Continuation" or "Continuation-in-part":	*in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;*
(vi) if there are more than three earlier applications whose priority is claimed:	*in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI.*
2. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning non-prejudicial disclosures or exceptions to lack of novelty: ▲ Continuation of Box No. IV	*in such case, write "Statement Concerning Non-Prejudicial Disclosures or Exceptions to Lack of Novelty" and furnish that statement below.*

RO

PURVIS, William Michael Cameron
COTTER, Ivan John
PILCH, Adam John Michael
CRISP, David Norman
ROBINSON, Nigel Alexander Julian
HARRIS, Ian Richard
HARDING, Charles Thomas
TURNER, James Arthur
PRICE, Paul Anthony King
PRATT, Richard Wilson
MALLALIEU, Catherine Louise
HOLMES, Miles Keeton
HORNER, David Richard
MASCHIO, Antonio
NACHSHEN, Neil Jacob
POTTER, Julian Mark

Box No. VI PRIORITY CLAIM Further priority claims are indicated in the Supplemental Box ☐

The priority of the following earlier application(s) is hereby claimed:

Country (in which, or for which, the application was filed)	Filing Date (day/month/year)	Application No.	Office of filing (only for regional or international application)
item (1) United Kingdom	(06.05.97)▲ ▲06 May 1997	9709161.5	
item (2)			
item (3)			

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present international application is the receiving Office (a fee may be required):

☐ The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s) : _____

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):* ISA / EP▲

Earlier search *Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request:*

Country (or regional Office): Date *(day/month/year)*: Number:

Box No. VIII CHECK LIST

This international application contains the following number of sheets:

1. request	5	sheets
2. description	48	sheets
3. claims	3	sheets
4. abstract	1	sheets
5. drawings		sheets
Total	57	sheets

This international application is accompanied by the item(s) marked below:

1. ☐ separate signed power of attorney
2. ☐ copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☐ priority document(s) *identified in Box No. VI as item(s):*
5. ☐ fee calculation sheet
6. ☐ separate indications concerning deposited microorganisms
7. ☐ nucleotide and/or amino acid sequence listing (diskette)
8. ☒ other *(specify)*: Letter

Figure No. _____ of the drawings (if any) should accompany the abstract when it is published.

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

.....

C T HARDING - AUTHORISED AGENT

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1. Date of actual receipt of the purported international application: 06 MAY 1998 06 05 98
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority specified by the applicant: ISA /
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A PROCESS OF PREPARING AN ANTI-OXIDANT

The present invention relates to a process of preparing an anti-oxidant.

An anti-oxidant prevents, inhibits or reduces the oxidation rate of an oxidisable medium. In particular, anti-oxidants are used for the preservation of food, especially when the food is or comprises a fat. Typical chemical anti-oxidants include aromatic amines, substituted phenols and sulphur compounds. Examples of anti-oxidants for food products are polyvinylpolypyrrolidone, dithiothreitol, sulphur dioxide, synthetic γ-tocopherol, δ-tocopherol, L-ascorbic acid, sodium L-ascorbate, calcium L-ascorbate, ascorbyl palmitate, propyl gallate, octyl gallate, dodecyl gallate, lecithin, diphenylamine ethoxyquin and butylated hydroxytoluene. Two commonly used anti-oxidants are GRINDOX 142 (obtained from Danisco A/S) and GRINDOX 1029 (obtained from Danisco A/S).

Typically, anti-oxidants are added to foodstuffs, such as beverages.

For example, anti-oxidants are used in the preparation of alcoholic beverages such as beer, cider, ale etc.. In particular, there is a wide spread use of anti-oxidants in the preparation of wine. In this regard, Butzke and Bisson in Agro-Food-Industry Hi-Tech (July/August 1996 pages 26-30) present a review of wine manufacture.

According to Butzke and Bisson (*ibid*):

> *"Wine is the product of the natural fermentation of grape must or juice. In the case of red wine, the skins are present during the initial fermentation to allow extraction of pigment and important flavour and aroma constituents from the skin. The term "must" refers to the crushed whole grapes. In the case of white wine production, skins are removed prior to fermentation and only the juice is retained and processed. ....*

Grapes are harvested and brought directly to the winery from the field. The grapes are then crushed at the winery and the must either transferred to a tank for fermentation (red wine) or pressed to separate juice from the skin and seeds (white wine). In this latter case, the juice is then transferred to a tank for fermentation. The tanks may either be inoculated with a commercial wine strain of Saccharomyces or allowed to undergo a natural or uninoculated fermentation. In a natural fermentation, Saccharomyces cells are greatly outnumbered by wild (non-Saccharomyces) yeast and bacteria at the beginning of fermentation. By the end of the fermentation Saccharomyces is the dominant and most often only organism isolateable. Inoculation with a commercial wine strain or with fermenting juice or must changes the initial ratio of the numbers of different microorganisms, allowing Saccharomyces to dominate the fermentation much earlier.

The metabolic activity of microorganisms in wine results in the production of aroma and flavour compounds some of which are highly objectionable to the consumer and all of which are distinct from the compounds responsible for the varietal character of the wine. .... Sulphur dioxide addition prevents chemical oxidation reactions and in this sense is an important stabilizer of the natural grape aroma and flavour. It may be added to the must or juice to preserve flavour, not necessarily as an antimicrobial agent. However, its antimicrobial activity must be considered when choosing a strain to be genetically modified for wine production."

Hence, potentially harmful chemicals - such as sulphur dioxide - are used in wine manufacture.

The present invention seeks to overcome any problems associated with the prior art methods of preparing foodstuffs with antioxidants.

According to a first aspect of the present invention there is provided a process of preparing a medium that comprises an anti-oxidant and at least one other component, the process comprising preparing *in situ* in the medium the anti-oxidant; and wherein the anti-oxidant is prepared from a glucan by use of recombinant DNA techniques.

According to a second aspect of the present invention there is provided a process of preparing a medium that comprises an anti-oxidant and at least one other component, the process comprising preparing *in situ* in the medium the anti-oxidant; and wherein the anti-oxidant is prepared by use of a recombinant glucan lyase.

According to a third aspect of the present invention there is provided a medium prepared by the process according to the present invention.

Other aspects of the present invention include:

Use of anhydrofructose as an anti-oxidant for a medium comprising at least one other component, wherein the anhydrofructose is prepared *in situ* in the medium.

Use of anhydrofructose as a means for imparting or improving stress tolerance in a plant, wherein the anhydrofructose is prepared *in situ* in the plant.

Use of anhydrofructose as a means for imparting or improving the transformation of a grape, wherein the anhydrofructose is prepared *in situ* in the grape.

Use of anhydrofructose as a means for increasing antioxidant levels in a foodstuff (preferably a fruit or vegetable, more preferably a fresh fruit or a fresh vegetable), wherein the anhydrofructose is prepared *in situ* in the foodstuff.

Use of anhydrofructose as a pharmaceutical in a foodstuff, wherein the anhydrofructose is prepared *in situ* in the foodstuff.

A method of administering a foodstuff comprising anhydrofructose, wherein the anhydrofructose is in a pharmaceutically acceptable amount and acts as a pharmaceutical; and wherein the anhydrofructose has been prepared *in situ* in the foodstuff.

Use of anhydrofructose as a nutraceutical in a foodstuff, wherein the anhydrofructose is prepared *in situ* in the foodstuff.

A method of administering a foodstuff comprising anhydrofructose, wherein the anhydrofructose is in a nutraceutically acceptable amount and acts as a nutraceutical; and wherein the anhydrofructose has been prepared *in situ* in the foodstuff.

Use of glucan lyase as a means for imparting or improving stress tolerance in a plant, wherein the glucan lyase is prepared *in situ* in the plant.

Use of glucan lyase as a means for imparting or improving the transformation of a grape, wherein the glucan lyase is prepared *in situ* in the grape.

Use of glucan lyase as a means for increasing antioxidant levels in a foodstuff (preferably a fruit or vegetable, more preferably a fresh fruit or a fresh vegetable), wherein the glucan lyase is prepared *in situ* in the foodstuff.

Use of glucan lyase in the preparation of a pharmaceutical in a foodstuff, wherein the glucan lyase is prepared *in situ* in the foodstuff.

A method of administering a foodstuff comprising an antioxidant, wherein the antioxidant is in a pharmaceutically acceptable amount and acts as a pharmaceutical; and wherein the antioxidant has been prepared *in situ* in the

foodstuff from a glucan lyase.

Use of glucan lyase in the preparation of a nutraceutical in a foodstuff, wherein the glucan lyase is prepared *in situ* in the foodstuff.

A method of administering a foodstuff comprising an antioxidant, wherein the antioxidant is in a nutraceutically acceptable amount and acts as a nutraceutical; and wherein the antioxidant has been prepared *in situ* in the foodstuff from a glucan lyase.

Use of a nucleotide sequence coding for a glucan lyase as a means for imparting or improving stress tolerance in a plant, wherein the nucleotide sequence is expressed *in situ* in the plant.

Use of a nucleotide sequence coding for a glucan lyase as a means for imparting or improving the transformation of a grape, wherein the nucleotide sequence is expressed *in situ* in the grape.

Use of a nucleotide sequence coding for a glucan lyase as a means for increasing antioxidant levels in a foodstuff (preferably a fruit or vegetable, more preferably a fresh fruit or a fresh vegetable), wherein the nucleotide sequence is expressed *in situ* in the foodstuff.

Use of a nucleotide sequence coding for a glucan lyase as a means for creating a pharmaceutical in a foodstuff, wherein the nucleotide sequence is expressed *in situ* in the foodstuff.

A method of administering a foodstuff comprising an antioxidant, wherein the antioxidant is in a pharmaceutically acceptable amount and acts as a pharmaceutical; and wherein the antioxidant has been prepared *in situ* in the foodstuff by means of a nucleotide sequence coding for a glucan lyase.

Use of a nucleotide sequence coding for a glucan lyase as a means for creating a nutraceutical in a foodstuff, wherein the nucleotide sequence is expressed *in situ* in the foodstuff.

A method of administering a foodstuff comprising an antioxidant, wherein the antioxidant is in a nutraceutically acceptable amount and acts as a nutraceutical; and wherein the antioxidant has been prepared *in situ* in the foodstuff by means of a nucleotide sequence coding for a glucan lyase.

The term "nutraceutical" means a compound that is capable of acting as a nutrient (i.e. it is suitable for, for example, oral administration) as well as being capable of exhibiting a pharmaceutical effect and/or cosmetic effect.

In contrast to the usual practice of adding anti-oxidants media, such as foodstuffs, we have now found that particular anti-oxidants can be prepared *in situ* in the medium.

The *in situ* preparation of anti-oxidants is particularly advantageous in that less, or even no, additional anti-oxidants need be added to the medium, such as a food product.

The present invention is also believed to be advantageous as it provides a means of improving stress tolerance of plants.

The present invention is also advantageous as it provides a means for viably transforming grape.

The present invention is further advantageous in that it enables the levels of antioxidants in foodstuffs to be elevated. This may have beneficial health implications. In this regard, recent reports (e.g. Biotechnology Newswatch April 21 1997 "*Potent Antioxidants, as strong as those in fruit, found in coffee*" by Marjorie Shaffer) suggest that antioxidants have a pharmaceutical benefit, for example in preventing or suppressing cancer formation.

General *in situ* preparation of antioxidants in plants has been previously reviewed by Badiani *et al* in Agro-Food-Industry Hi-Tech (March/April 1996 pages 21-26). It is to be noted, however, that this review does not mention preparing *in situ* antioxidants from a glucan, let alone by use of a recombinant glucan lyase.

Preferably, the glucan comprises α-1,4 links.

Preferably, the glucan is starch or a unit of starch.

Preferably, the glucan is a substrate for a recombinant enzyme such that contact of the glucan with the recombinant enzyme yields the anti-oxidant.

Preferably, the enzyme is a glucan lyase.

Preferably, the enzyme is an α-1,4-glucan lyase.

Preferably, the enzyme comprises any one of the sequences shown as SEQ ID Nos 1-6, or a variant, homologue or fragment thereof.

Preferably, the enzyme is any one of the sequences shown as SEQ ID Nos 1-6.

Preferably, the enzyme is encoded by a nucleotide sequence comprising any one of the sequences shown as SEQ ID Nos 7-12, or a variant, homologue or fragment thereof.

Preferably, the enzyme is encoded by a nucleotide sequence having any one of the sequences shown as SEQ ID Nos 7-12.

Preferably, the anti-oxidant is anhydrofructose.

Preferably, the anti-oxidant is 1,5-D-anhydrofructose.

Preferably, the medium is, or is used in the preparation of, a foodstuff.

Preferably, the foodstuff is a beverage.

Preferably, the beverage is an alcoholic beverage.

Preferably, the beverage is a wine.

Preferably, the anti-oxidant is prepared *in situ* in the component and is then released into the medium.

Preferably, the component is a plant or a part thereof.

Preferably, the component is all or part of a cereal or a fruit.

Preferably, the component is all or part of a grape.

The medium may be used as or in the preparation of a foodstuff, which includes beverages. In the alternative, the medium may be for use in polymer chemistry. In this regard, the *in situ* generated anti-oxidants could therefore act as oxygen scavengers during, for example, the synthesis of polymers, such as the synthesis of bio-degradable plastic.

In accordance with the present invention, the anti-oxidant (preferably anhydrofructose) is prepared *in situ* in the medium. In other words, the antioxidant (preferably anhydrofructose) that is prepared *in situ* in the medium is used as an anti-oxidant in the medium. In one emdodiment, the antioxidant (preferably anhydrofructose) that is prepared *in situ* in the medium is used as the main anti-oxidant in the medium.

The term "*in situ* in the medium" as used herein includes the anti-oxidant being prepared by action of a recombinant enzyme expressed by the component on a glucan - which glucan is a substrate for the enzyme. The term also includes the anti-oxidant

being prepared by action of a recombinant enzyme expressed by the component on a glucan - which glucan is a substrate for the enzyme - within the component and the subsequent generation of the anti-oxidant. The term also includes the recombinant enzyme being expressed by the component and then being released into the medium, which enzyme acts on a glucan - which glucan is a substrate for the enzyme - present in the medium to form the anti-oxidant in the medium. The term also covers the presence or addition of another component to the medium, which component then expresses a recombinant nucleotide sequence which results in exposure of part or all of the medium to an anti-oxidant, which anti-oxidant may be a recombinant enzyme or a recombinant protein expressed and released by the other component, or it may be a product of a glucan - which glucan is a substrate for the enzyme - within the medium that has been exposed to the recombinant enzyme or the recombinant protein.

The term "by use of recombinant DNA techniques" as used herein includes the anti-oxidant being any obtained by use of a recombinant enzyme or a recombinant protein, which enzyme or protein acts on the glucan. The term also includes the anti-oxidant being any obtained by use of an enzyme or protein, which enzyme or protein acts on a recombinant glucan.

The term "starch" in relation to the present invention includes native starch, degraded starch, modified starch, including its components amylose and amylopectin, and the glucose units thereof.

The terms "variant", "homologue" or "fragment" in relation to the enzyme include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) amino acid from or to the sequence providing the resultant amino acid sequence has α-glucan lyase activity, preferably having at least the same activity of any one of the enzymes shown as SEQ ID No. 1-6. In particular, the term "homologue" covers homology with respect to structure and/or function providing the resultant enzyme has α-glucan lyase activity. With respect to sequence homology, preferably there is at least 75%, more preferably at least 85%, more preferably at least 90% homology to any one of the sequences shown as SEQ ID No.s 1-6. More

preferably there is at least 95%, more preferably at least 98%, homology to any one of the sequences shown as SEQ ID No. 1-6.

The terms "variant", "homologue" or "fragment" in relation to the nucleotide sequence coding for the enzyme include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the sequence providing the resultant nucleotide sequence codes for an enzyme having α-glucan lyase activity, preferably having at least the same activity of any one of the enzymes shown as SEQ ID No. 1-6. In particular, the term "homologue" covers homology with respect to structure and/or function providing the resultant nucleotide sequence codes for an enzyme having α-glucan lyase activity. With respect to sequence homology, preferably there is at least 75%, more preferably at least 85%, more preferably at least 90% homology to any one of the sequences shown as SEQ ID No. 7-12. More preferably there is at least 95%, more preferably at least 98%, homology to any one of the sequences shown as SEQ ID No. 7-12.

The above terms are synonymous with allelic variations of the sequences.

The present invention also covers nucleotide sequences that can hybridise to the nucleotide sequence of the present invention.

The term "nucleotide" in relation to the present invention includes cDNA.

According to the present invention there is therefore provided a method of preparing *in situ* in an oxidisable medium an anti-oxidant. In a preferred embodiment, the anti-oxidant is anhydrofructose, more preferably 1,5-D-anhydrofructose. 1,5-D-anhydrofructose has been chemically synthesised (Lichtenthaler in Tetrahedron Letters Vol 21 pp 1429-1432). 1,5-D-anhydrofructose is further discussed in WO 95/10616, WO 95/10618 and GB-B-2294048.

The main advantages of using 1,5-D-anhydrofructose as an anti-oxidant are that it is a natural product, it is non-metabolisable, it is easy to manufacture, it is water-soluble, and it is generally non-toxic.

According to WO 95/10616, WO 95/10618 and GB-B-2294048, 1,5-D-anhydrofructose may be prepared by the enzymatic modification of substrates based on α-1,4-glucan by use of the enzyme α-1,4-glucan lyase. A typical α-1,4-glucan based substrate is starch.

Today, starches have found wide uses in industry mainly because they are cheap raw materials. There are many references in the art to starch. For example, starch is discussed by Salisbury and Ross in Plant Physiology (Fourth Edition, 1991, Published by Wadsworth Publishing Company - especially section 11.7). In short, however, starch is one of the principal energy reserves of plants. It is often found in colourless plastids (amyloplasts), in storage tissue and in the stroma of chloroplasts in many plants. Starch is a polysaccharide carbohydrate. It comprises two main components: amylose and/or amylopectin. Both amylose and/or amylopectin consist of straight chains of α(1,4)-linked glucose units (ie glycosyl residues) but in addition amylopectin includes α(1,6) branched glucose units.

Some of the glucan lyases discussed in WO 95/10616 and WO 95/10618 that are suitable for producing 1,5-D-anhydrofructose from starch are shown as SEQ I.D. No.s 1-4. Some of the glucan lyases discussed in GB-B-2294048 that are suitable for producing 1,5-D-anhydrofructose from starch are shown as SEQ I.D. No.s 5-6.

Some of the nucleotide sequences coding for glucan lyases discussed in WO 95/10616 and WO 95/10618 that are suitable for producing 1,5-D-anhydrofructose from starch are shown as SEQ I.D. No.s 7-10. Some of the nucleotide sequences coding for glucan lyases discussed in GB-B-2294048 that are suitable for producing 1,5-D-anhydrofructose from starch are shown as SEQ I.D. No.s 11-12.

A further glucan lyase is discussed in WO 94/09122.

The recombinant nucleotide sequences coding for the enzyme may be cloned from sources such as a fungus, preferably *Morchella costata or Morchella vulgaris,* or from a fungally infected algae, preferably *Gracilariopsis lemaneiformis*, or from algae lone, preferably *Gracilariopsis lemaneiformis*.

In a preferred embodiment, the 1,5-D-anhydrofructose is prepared *in situ* by treating an α-1,4-glucan with a recombinant α-1,4-glucan lyase, such as any one of those presented as SEQ I.D. No.s 1-6.

Detailed commentary on how to prepare the enzymes shown as sequences SEQ I.D. No.s 1-6 may be found in the teachings of WO 95/10616, WO 95/10618 and GB-B-2294048. Likewise, detailed commentary on how to isolate and clone the nucleotide sequences SEQ I.D. No.s 7-12 may be found in the teachings of WO 95/10616, WO 95/10618 and GB-B-2294048.

If the glucan contains links other than and in addition to the α-1,4- links the recombinant α-1,4-glucan lyase can be used in conjunction with a suitable reagent that can break the other links - such as a recombinant hydrolase - preferably a recombinant glucanohydrolase.

General teachings of recombinant DNA techniques may be found in Sambrook,J., Fritsch, E.F., Maniatis T. (Editors) Molecular Cloning. A laboratory manual. Second edition. Cold Spring Harbour Laboratory Press. New York 1989.

In order to express a nucleotide sequence, the host organism can be a prokaryotic or a eukaryotic organism. Examples of suitable prokaryotic hosts include *E. coli* and *Bacillus subtilis*. Teachings on the transformation of prokaryotic hosts is well documented in the art, for example see Sambrook *et al* (Molecular Cloning: A Laboratory Manual, 2nd edition, 1989, Cold Spring Harbor Laboratory Press). If a prokaryotic host is used then the gene may need to be suitably modified before transformation - such as by removal of introns.

In one embodiment, the host organism can be of the genus *Aspergillus*, such as *Aspergillus niger*. A transgenic *Aspergillus* can be prepared by following the teachings of Rambosek, J. and Leach, J. 1987 (Recombinant DNA in filamentous fungi: Progress and Prospects. CRC Crit. Rev. Biotechnol. 6:357-393), Davis R.W. 1994 (Heterologous gene expression and protein secretion in *Aspergillus*. In: Martinelli S.D., Kinghorn J.R.(Editors) *Aspergillus*: 50 years on. Progress in industrial microbiology vol 29. Elsevier Amsterdam 1994. pp 525-560), Ballance, D.J. 1991 (Transformation systems for Filamentous Fungi and an Overview of Fungal Gene structure. In: Leong, S.A., Berka R.M. (Editors) Molecular Industrial Mycology. Systems and Applications for Filamentous Fungi. Marcel Dekker Inc. New York 1991. pp 1-29) and Turner G. 1994 (Vectors for genetic manipulation. In: Martinelli S.D., Kinghorn J.R.(Editors) *Aspergillus*: 50 years on. Progress in industrial microbiology vol 29. Elsevier Amsterdam 1994. pp. 641-666). However, the following commentary provides a summary of those teachings for producing transgenic *Aspergillus*.

For almost a century, filamentous fungi have been widely used in many types of industry for the production of organic compounds and enzymes. For example, traditional japanese koji and soy fermentations have used *Aspergillus sp*. Also, in this century *Aspergillus niger* has been used for production of organic acids particular citric acid and for production of various enzymes for use in industry.

There are two major reasons why filamentous fungi have been so widely used in industry. First filamentous fungi can produce high amounts of extracelluar products, for example enzymes and organic compounds such as antibiotics or organic acids. Second filamentous fungi can grow on low cost substrates such as grains, bran, beet pulp etc. The same reasons have made filamentous fungi attractive organisms as hosts for heterologous expression of recombinant enzymes according to the present invention.

In order to prepare the transgenic *Aspergillus*, expression constructs are prepared by inserting a requisite nucleotide sequence into a construct designed for expression in filamentous fungi.

Several types of constructs used for heterologous expression have been developed. These constructs can contain a promoter which is active in fungi. Examples of promoters include a fungal promoter for a highly expressed extracelluar enzyme, such as the glucoamylase promoter or the α-amylase promoter. The nucleotide sequence can be fused to a signal sequence which directs the protein encoded by the nucleotide sequence to be secreted. Usually a signal sequence of fungal origin is used. A terminator active in fungi ends the expression system.

Another type of expression system has been developed in fungi where the nucleotide sequence can be fused to a smaller or a larger part of a fungal gene encoding a stable protein. This can stabilize the protein encoded by the nucleotide sequence. In such a system a cleavage site, recognized by a specific protease, can be introduced between the fungal protein and the protein encoded by the nucleotide sequence, so the produced fusion protein can be cleaved at this position by the specific protease thus liberating the protein encoded by the nucleotide sequence. By way of example, one can introduce a site which is recognized by a KEX-2 like peptidase found in at least some *Aspergilli*. Such a fusion leads to cleavage *in vivo* resulting in protection of the expressed product and not a larger fusion protein.

Heterologous expression in *Aspergillus* has been reported for several genes coding for bacterial, fungal, vertebrate and plant proteins. The proteins can be deposited intracellularly if the nucleotide sequence is not fused to a signal sequence. Such proteins will accumulate in the cytoplasm and will usually not be glycosylated which can be an advantage for some bacterial proteins. If the nucleotide sequence is equipped with a signal sequence the protein will accumulate extracelluarly.

With regard to product stability and host strain modifications, some heterologous proteins are not very stable when they are secreted into the culture fluid of fungi.

Most fungi produce several extracelluar proteases which degrade heterologous proteins. To avoid this problem special fungal strains with reduced protease production have been used as host for heterologous production.

For the transformation of filamentous fungi, several transformation protocols have been developed for many filamentous fungi (Ballance 1991, *ibid*). Many of them are based on preparation of protoplasts and introduction of DNA into the protoplasts using PEG and Ca^{2+} ions. The transformed protoplasts then regenerate and the transformed fungi are selected using various selective markers. Among the markers used for transformation are a number of auxotrophic markers such as *argB*, *trpC*, *niaD* and *pyrG*, antibiotic resistance markers such as benomyl resistance, hygromycin resistance and phleomycin resistance. A commonly used transformation marker is the *amdS* gene of *A. nidulans* which in high copy number allows the fungus to grow with acrylamide as the sole nitrogen source.

In another embodiment the transgenic organism can be a yeast. In this regard, yeast have also been widely used as a vehicle for heterologous gene expression. The species *Saccharomyces cerevisiae* has a long history of industrial use, including its use for heterologous gene expression. Expression of heterologous genes in *Saccharomyces cerevisiae* has been reviewed by Goodey *et al* (1987, Yeast Biotechnology, D R Berry *et al*, eds, pp 401-429, Allen and Unwin, London) and by King *et al* (1989, Molecular and Cell Biology of Yeasts, E F Walton and G T Yarronton, eds, pp 107-133, Blackie, Glasgow).

For several reasons *Saccharomyces cerevisiae* is well suited for heterologous gene expression. First, it is non-pathogenic to humans and it is incapable of producing certain endotoxins. Second, it has a long history of safe use following centuries of commercial exploitation for various purposes. This has led to wide public acceptability. Third, the extensive commercial use and research devoted to the organism has resulted in a wealth of knowledge about the genetics and physiology as well as large-scale fermentation characteristics of *Saccharomyces cerevisiae*.

A review of the principles of heterologous gene expression in *Saccharomyces cerevisiae* and secretion of gene products is given by E Hinchcliffe E Kenny (1993, "Yeast as a vehicle for the expression of heterologous genes", Yeasts, Vol 5, Anthony H Rose and J Stuart Harrison, eds, 2nd edition, Academic Press Ltd.).

Several types of yeast vectors are available, including integrative vectors, which require recombination with the host genome for their maintenance, and autonomously replicating plasmid vectors.

In order to prepare the transgenic *Saccharomyces*, expression constructs are prepared by inserting the nucleotide sequence into a construct designed for expression in yeast. Several types of constructs used for heterologous expression have been developed. The constructs contain a promoter active in yeast fused to the nucleotide sequence, usually a promoter of yeast origin, such as the GAL1 promoter, is used. Usually a signal sequence of yeast origin, such as the sequence encoding the SUC2 signal peptide, is used. A terminator active in yeast ends the expression system.

For the transformation of yeast several transformation protocols have been developed. For example, a transgenic *Saccharomyces* can be prepared by following the teachings of Hinnen *et al* (1978, Proceedings of the National Academy of Sciences of the USA 75, 1929); Beggs, J D (1978, Nature, London, 275, 104); and Ito, H *et al* (1983, J Bacteriology 153, 163-168).

The transformed yeast cells are selected using various selective markers. Among the markers used for transformation are a number of auxotrophic markers such as LEU2, HIS4 and TRP1, and dominant antibiotic resistance markers such as aminoglycoside antibiotic markers, eg G418.

Another host organism is a plant. In this regard, the art is replete with references for preparing transgenic plants. Two documents that provide some background commentary on the types of techniques that may be employed to prepare transgenic plants are EP-B-0470145 and CA-A-2006454 - some of which commentary is

presented below.

The basic principle in the construction of genetically modified plants is to insert genetic information in the plant genome so as to obtain a stable maintenance of the inserted genetic material.

Several techniques exist for inserting the genetic information, the two main principles being direct introduction of the genetic information and introduction of the genetic information by use of a vector system. A review of the general techniques may be found in articles by Potrykus (Annu Rev Plant Physiol Plant Mol Biol [1991] 42:205-225) and Christou (Agro-Food-Industry Hi-Tech March/April 1994 17-27).

Thus, in one aspect, the present invention relates to a vector system which carries a recombinant nucleotide sequence and which is capable of introducing the nucleotide sequence into the genome of an organism, such as a plant, and wherein the nucleotide sequence is capable of preparing *in situ* an anti-oxidant.

The vector system may comprise one vector, but it can comprise at least two vectors. In the case of two vectors, the vector system is normally referred to as a binary vector system. Binary vector systems are described in further detail in Gynheung An et al. (1980), Binary Vectors, *Plant Molecular Biology Manual A3*, 1-19.

One extensively employed system for transformation of plant cells with a given promoter or nucleotide sequence or construct is based on the use of a Ti plasmid from *Agrobacterium tumefaciens* or a Ri plasmid from *Agrobacterium rhizogenes* (An et al. (1986), *Plant Physiol.* 81, 301-305 and Butcher D.N. *et al.* (1980), *Tissue Culture Methods for Plant Pathologists,* eds.: D.S. Ingrams and J.P. Helgeson, 203-208).

Several different Ti and Ri plasmids have been constructed which are suitable for the construction of the plant or plant cell constructs described above.

The nucleotide sequence of the present invention should preferably be inserted into

the Ti-plasmid between the border sequences of the T-DNA or adjacent a T-DNA sequence so as to avoid disruption of the sequences immediately surrounding the T-DNA borders, as at least one of these regions appear to be essential for insertion of modified T-DNA into the plant genome.

As will be understood from the above explanation, if the organism is a plant, then the vector system of the present invention is preferably one which contains the sequences necessary to infect the plant (e.g. the *vir* region) and at least one border part of a T-DNA sequence, the border part being located on the same vector as the genetic construct. Preferably, the vector system is an *Agrobacterium tumefaciens* Ti-plasmid or an *Agrobacterium rhizogenes* Ri-plasmid or a derivative thereof, as these plasmids are well-known and widely employed in the construction of transgenic plants, many vector systems exist which are based on these plasmids or derivatives thereof.

In the construction of a transgenic plant the nucleotide sequence or construct or vector of the present invention may be first constructed in a microorganism in which the vector can replicate and which is easy to manipulate before insertion into the plant. An example of a useful microorganism is *E. coli.*, but other microorganisms having the above properties may be used. When a vector of a vector system as defined above has been constructed in *E. coli.* it is transferred, if necessary, into a suitable *Agrobacterium* strain, e.g. *Agrobacterium tumefaciens*. The Ti-plasmid harbouring the first nucleotide sequence or construct of the invention is thus preferably transferred into a suitable *Agrobacterium* strain, e.g. *A. tumefaciens*, so as to obtain an *Agrobacterium* cell harbouring the promoter or nucleotide sequence or construct of the invention, which DNA is subsequently transferred into the plant cell to be modified.

As reported in CA-A-2006454, a large number of cloning vectors are available which contain a replication system in *E. coli* and a marker which allows a selection of the transformed cells. The vectors contain for example pBR322, the pUC series, the M13 mp series, pACYC 184 etc. In this way, the promoter or nucleotide or construct of the present invention can be introduced into a suitable restriction position

in the vector. The contained plasmid is used for the transformation in *E.coli*. The *E.coli* cells are cultivated in a suitable nutrient medium and then harvested and lysed. The plasmid is then recovered and then analysed - such as by any one or more of the following techniques: sequence analysis, restriction analysis, electrophoresis and further biochemical-molecular biological methods. After each manipulation, the used DNA sequence can be restricted or selectively amplified by PCR techniques and connected with the next DNA sequence. Each sequence can be cloned in the same or different plasmid.

After each introduction method of the nucleotide sequence or construct or vector according to the present invention in the plants the presence and/or insertion of further DNA sequences may be necessary. If, for example, for the transformation the Ti- or Ri-plasmid of the plant cells is used, at least the right boundary and often however the right and the left boundary of the Ti- and Ri-plasmid T-DNA, as flanking areas of the introduced genes, can be connected. The use of T-DNA for the transformation of plant cells has been intensively studied and is described in EP-A-120516; Hoekema, in: The Binary Plant Vector System Offset-drukkerij Kanters B.B., Alblasserdam, 1985, Chapter V; Fraley, *et al.*, Crit. Rev. Plant Sci., 4:1-46; and An et al., EMBO J. (1985) 4:277-284.

Direct infection of plant tissues by *Agrobacterium* is a simple technique which has been widely employed and which is described in Butcher D.N. et al. (1980), *Tissue Culture Methods for Plant Pathologists,* eds.: D.S. Ingrams and J.P. Helgeson, 203-208. For further teachings on this topic see Potrykus (Annu Rev Plant Physiol Plant Mol Biol [1991] 42:205-225) and Christou (Agro-Food-Industry Hi-Tech March/April 1994 17-27). With this technique, infection of a plant may be done on a certain part or tissue of the plant, i.e. on a part of a leaf, a root, a stem or another part of the plant.

Typically, with direct infection of plant tissues by *Agrobacterium* carrying the first nucleotide sequence or the construct, a plant to be infected is wounded, e.g. by cutting the plant with a razor or puncturing the plant with a needle or rubbing the

plant with an abrasive. The wound is then inoculated with the *Agrobacterium*. The inoculated plant or plant part is then grown on a suitable culture medium.

When plant cells are constructed, these cells are grown and, optionally, maintained in a medium according to the present invention following well-known tissue culturing methods - such as by culturing the cells in a suitable culture medium supplied with the necessary growth factors such as amino acids, plant hormones, vitamins, etc, but wherein the culture medium comprises a component according to the present invention. Regeneration of the transformed cells into genetically modified plants may be accomplished using known methods for the regeneration of plants from cell or tissue cultures, for example by selecting the transformed shoots and by subculturing the shoots on a medium containing the appropriate nutrients, plant hormones, etc.

Further teachings on plant transformation may be found in EP-A-0449375.

Reference may even be made to Spngstad *et al* (1995 Plant Cell Tissue Organ Culture **40** pp 1-15) as these authors present a general overview on transgenic plant construction.

In one embodiment, the plant is a grapevine. There are a number of teachings in the art on how to prepare transformed grapevines. For example, reference may be made to Baribault *et al* (J Exp Bot 41 (229) 1990 1045-1050), Baribault *et al* (Plant Cell Rep 8 (3) 1989 137-140), Scorza *et al* (J Am Soc Horticultural Science 121 (4) 1996 616-619), Kikkert *et al* (Plant Cell Reports 15 (5) 1996 311-316), Golles *et al* (Acta Hortic 1997 vol 447 Number: Horticultural Biotechnology in Vitro Culture and Breeding Pages 265-275), Gray and Scorza (WO-A-97/49277) and Simon Robinson *et al* (Conference abstracts and paper presented in Biotechnology - Food and Health for the 21st Century, Adelaide, Australia, 1998). By way of example Robinson *et al* (*ibid*) disclose a method for transforming grapevine wherein somatic embryos are induced on callus formed from another tissue and *Agrobacterium* infection is used to transfer target genes into the embryo tissue.

Further reference may be made to the teachings of Andrew Walker in Nature Biotechnology (Vol 14, May 1996, page 582) who states that:

> *"The grape, one of the most important fruit plants in the world, has been difficult to engineer because of its high levels of tannins and phenols, which interfere with cell culture and transformation; the compounds oxidize quickly and promote the decay of grape cells."*

In that same edition of Nature Biotechnology, Perl *et al* (pages 624-628) report on the use of the combination of polyvinylpolypyrrolidone and dithiothreitol to improve the viability of grape transformation during *Agrobacterium* infection.

Hence, the present invention provides an alternative means for transforming grape. In this regard, the antioxidant that is prepared *in situ* by a grape transformed in accordance with the present invention improves the viability of grape transformation during *Agrobacterium* infection.

Thus, according to one aspect of the present invention, there is provided the use of an antioxidant prepared *in situ* in order to effectively transform a grape.

In some instances, it is desirable for the recombinant enzyme or protein to be easily secreted into the medium to act as or to generate an anti-oxidant therein. In such cases, the DNA encoding the recombinant enzyme is fused to *inter alia* an appropriate signal sequence, an appropriate promoter and an appropriate terminator from the chosen host.

For example, for expression in *Aspergillus niger* the gpdA (from the Glyceraldehyde-3-phosphate dehydrogenase gene of *Aspergillus nidulans*) promoter and signal sequence is fused to the 5' end of the DNA encoding the mature lyase. The terminator sequence from the *A. niger* trpC gene is placed 3' to the gene (Punt, P.J. et al 1991 - (1991): J. Biotech. 17, 19-34). This construction is inserted into a vector containing a replication origin and selection origin for *E. coli* and a selection marker

for *A. niger*. Examples of selection markers for *A. niger* are the amdS gene, the argB gene, the pyrG gene, the hygB gene, the BmlR gene which all have been used for selection of transformants. This plasmid can be transformed into *A. niger* and the mature lyase can be recovered from the culture medium of the transformants. Eventually the construction could be transformed into a protease deficient strain to reduce the proteolytic degradation of the lyase in the medium (Archer D.B. et al 1992 -Biotechnol. Lett. 14, 357-362).

In addition, and as indicated above, aside from using *Aspergillus niger* as the host, there are other industrial important microorganisms which could be used as expression systems. Examples of these other hosts include: *Aspergillus oryzae, Aspergillus sp., Trichoderma sp., Saccharomyces cerevisiae, Kluyveromyces sp., Hansenula sp., Pichia sp., Bacillus subtilis, B. amyloliquefaciens, Bacillus sp., Streptomyces sp.* or *E. coli*.

In accordance with the present invention, a suitable marker or selection means may be introduced into the host that is to be transformed with the nucleotide sequence. Examples of suitable markers or selection means are described in any one of WO-A-93/05163, WO-A-94/20627, GB patent application No. 9702591.0 (filed 7 February 1997), GB patent application No. 9702576.1 (filed 7 February 1997), GB patent application No. 9702539.9 (filed 7 February 1997), GB patent application No. 9702510.0 (filed 7 February 1997) and GB patent application No. 9702592.8 (filed 7 February 1997).

In summation, the present invention relates to a process comprising preparing a medium that comprises an anti-oxidant and at least one other component, the process comprising preparing *in situ* in the medium the anti-oxidant; and wherein the anti-oxidant is prepared from a glucan by use of recombinant DNA techniques and/or the anti-oxidant is prepared by use of a recombinant glucan lyase.

In a preferred embodiment, the present invention relates to a process a process of preparing a medium that comprises an anti-oxidant and at least one other component. the process comprising preparing *in situ* in the medium the anti-oxidant; and wherein the anti-oxidant is prepared from a glucan by use of a recombinant glucan lyase.

In a more preferred embodiment, the present invention relates to a process of preparing a medium that comprises an anti-oxidant and at least one other component, the process comprising preparing *in situ* in the medium the anti-oxidant; wherein the anti-oxidant is prepared from a glucan by use of a recombinant glucan lyase; and wherein the anti-oxidant is anhydro-fructose.

The present invention will now be described only by way of example.

TRANSGENIC GRAPE

Transformed grapes are prepared following the teachings of Perl *et al* (*ibid*) but wherein the use of the combination of polyvinylpolypyrrolidone and dithiothreitol is optional. In these studies, the grapes are transformed with any one of the nucleotide sequences presented as SEQ ID No. 7-12. The transformation leads to *in situ* preparation of 1,5-D-anhydrofructose. The transformed grapes are beneficial for one or more of the reasons mentioned earlier.

Details on these studies are as follows.

Tissue-culture systems for transformation studies

The long term somatic embryogenic callus culture is developed from the vegetative tissues of anthers of *Vitis vinifera* CV Superior Seedless. Methods for another culture, induction of somatic embryogenesis and maintenance of embryogenic cultures, are previously described (Perl *et al*, 1995, Plant Sci **104**: 193-200). Briefly, embryogenic calli are maintained on solidified (0.25% gelrite) MS medium (Murashige and Skoog, 1962, Physiol Plant **15**: 473-497) supplemented with 6%

sucrose, 2 mg/L 2,4-diclorophenoxyacetic acid (2,4-D), 5 mg/L Indole-3-aspartic acid (IASP), 0.2 mg/L 6-benzyladenine (BAP) and 1 mg/L abscisic acid (ABA). Proembryogenic calli are induced by transferring the calli to MS medium supplemented with the same phytohormones, but 2,4-D is substituted with 2 mg/L 2-naphthoxyacetic acid (NOA). This stage is used for transformation experiments.

***Agrobacterium* strains**

For studying the sensitivity of grape embryogenic calli to the presence of different *Agrobacterium* strains, or for stable transformation experiments, cocultivation is attempted using the following *A tumefaciens* strains: EHA 101-p492 (Perl *et al*, 1993, Bio/Technology **11**:715-718); LBA 4404-pGPTV (Becker *et al*, 1992, Plant Mol Biol **20**: 1195-1197); and GVE 3101-pPCV91 (Vancanneyt *et al*, 1990, Mol Gen Genet **220**: 245-250). These strains contain the binary vectors conferring resistance to kanamycin (*nptII*), basta (*bar*) and hygromycin (*hpt*), respectively, all under the control of the nopalin-synthase (NOS) promoter and terminator. Bacteria are cultured with the proper antibiotics in liquid LB medium for 24 hours at 28°C at 200 rpm.

Cocultivation

For studying the sensitivity of grape embryogenic calli to different *Agrobacterium* strains, bacterial cultures with different optical densities (0.1-0.7 at 630 nm) are prepared from an overnight culture of *Agrobacterium* strains. Bacteria are centrifuged 5 minutes, 5000 rpm and resuspended in antibiotic free McCown's Woody Plant Medium (WPM) (Lloyd and McCown, 1981, Int Plant Prop Soc Proc **30**: 421-427). Three grams fresh weight of embryogenic calli (7 days after transfer to NOA containing medium) are resuspended in 10 ml of overnight cultured bacterial suspensions for 5 minutes, dry blotted and transferred to Petri dishes containing regeneration medium [basal WPM medium supplemented with thidiazuron (TDZ) (0.5 mg/L), Zeatin riboside (ZR) (0.5 mg/L), and sucrose (3%)]. The regeneration medium is solidified with gelrite (0.25% w/v) and the calli, after initial drainage of excess bacteria, are cocultivated in the dark at 25°C for different times (5 minutes

up to 7 days). For stable transformation experiments, inoculum (OD 0.6 at 630 nm) is prepared from an overnight culture of LBA 4404 or GVE 3101. Bacteria are centrifuged 5 minutes, 5000 rpm and resuspended in antibiotic-free WPM medium. Embryogenic calli (3g fresh weight) are resuspended in 10 ml of bacteria for 5 minutes, dry blotted and transferred to Petri dishes containing solidified (0.25% w/v) gelrite regeneration medium supplemented with different antioxidants. The calli are cocultivated for 48 hours in the dark at 25°C.

Selective culture

Following 48 hours of cocultivation, the embryogenic callus is maintained in the dark for 7 days on antioxidant containing regeneration medium. Subsequently, the calli are collected on a sterile metal screen and transferred to fresh WPM regeneration medium at 25 °C under 40 $\mu E/m^2/s$ (white fluorescent tubes). All regeneration media are supplemented with 400 mg/L claforan, 1.5 g/L malt extract and different selectable markers: kanamycin (50-500 mg/L), hygromycin (15 mg/L) and Basta (1-10 mg/L). Periodic increases in hygromycin concentration are used. The putative transformed calli are cultured on regeneration medium supplemented with 15 mg/L hygromycin. Every two weeks the regenerating calli are transferred to fresh medium supplemented with 20 and 25 mg/L hygromycin respectively. Control, untransformed grape calli are also cultured on selective media and are periodically exposed to increasing hygromycin concentrations. Green adventitious embryos, which developed on calli cultured for 8-10 weeks on selective regeneration medium, are transferred to germination medium. Embryo germination, rooting and subsequent plantlet development are induced on WPM as described (Perl *et al*, 1995, Plant Sci **104**: 193-200), supplemented with 25 mg/L hygromycin or 10 mg/L basta. Conversion of vitrified abnormal plantlets into normal-looking grape plantlets are obtained using solidified WPM medium supplemented with 0.1 mg/L NAA as described (Perl *et al*, 1995, Plant Sci **104**: 193-200).

TRANSGENIC POTATOES

General teachings on potato transformation may be found in our copending patent applications PCT/EP96/03053, PCT/EP96/03052 and PCT/EP94/01082 (the contents of each of which are incorporated herein by reference).

For the present studies, the following protocol is adopted.

Plasmid construction

The disarmed *Agrobacterium tumefaciens* strain LBA 4404, containing the helper *vir* plasmid pRAL4404 (Hoekema *et al*, 1983 Nature **303** pp 179-180), is cultured on YMB agar ($K_2HPO_4.3H_2O$ 660 mg l^{-1}, $MgSO_4$ 200 mg l^{-1}, NaCl 100 mg l^{-1}, mannitol 10 g l^{-1}, yeast extract 400 mg l^{-1}, 0.8% w/v agar, pH 7.0) containing 100 mg l^{-1} rifampicin and 500 mg l^{-1} streptomycin sulphate. Transformation with pVICTOR IV GNG E35S *nagB* IV2' or pVICTOR IV GNG rbc *nagB* IV2' or pVICTOR IV GNG E35S *nagB*' (which correspond to each of pVICTOR IV GNG E35S *nagB* IV2 or pVICTOR IV GNG rbc *nagB* IV2 or pVICTOR IV GNG E35S *nagB* but wherein each of those plasmids also contains any one of the nucleotide sequences shown as SEQ ID No.s. 7-12 operatively linked to a functional promoter) is accomplished using the freeze-thaw method of Holters *et al* (1978 Mol Gen Genet **163** 181-187) and transformants are selected on YMB agar containing 100 mg l^{-1} rifampicin and 500 mg l^{-1} streptomycin, and 50 mg l^{-1} gentamycin sulphate.

Transformation of plants

Shoot cultures of *Solanum tuberosum* cv Saturna are maintained on LS agar containing Murashige Skoog basal salts (Sigma M6899) (Murashige and Skoog, 1965, Physiol Plant **15** 473-497) with 2 μM silver thiosulphate, and nutrients and vitamins as described by Linsmaier and Skoog (1965 Physiol Plant **18** 100-127). Cultures are maintained at 25°C with a 16h daily photoperiod. After approximately 40 days, subculturing is performed during which leaves are removed, and the shoots cut into

mononodal segments of approximately 8 mm length.

Shoot cultures of approximately 40 days maturity (5-6 cm height) are cut into 8 mm internodal segments which are placed into liquid LS-medium containing *Agrobacterium tumefaciens* transformed with pVICTOR IV GNG E35S *nagB* IV2' or pVICTOR IV GNG rbc *nagB* IV2' or pVICTOR IV GNG E35S *nagB'* (A_{660} = 0.5, pathlength 1 cm). Following incubation at room temperature for 30 minutes, the segments are dried by blotting on to sterile filter paper and transferred to LS agar (0.8% w/v containing 2 mg l^{-1} 2,4-D and 500 μg l^{-1} trans-zeatin. The explants are covered with filter paper, moistened with LS medium, and covered with a cloth for three days at 25°C. Following this treatment, the segments are washed with liquid LS medium containing 800 mg l^{-1} carbenicillin, and transferred on to LS agar (0.8% w/v) containing 1 mg l^{-1} trans-zeatin, 100 μg l^{-1} gibberellic acid (GA3), with sucrose (eg 7.5 g l^{-1}) and glucosamine (eg 2.5 g l^{-1}) as the selection agent.

The segments are sub-cultured to fresh substrate each 3-4 weeks. In 3 to 4 weeks, shoots develop from the segments and the formation of new shoots continues for 3-4 months.

Rooting of regenerated shoots

The regenerated shoots are transferred to rooting substrate composed of LS-substrate, agar (8 g/l) and carbenicillin (800 mg/l).

The transgenic plants may be verified by performing a GUS assay on the co-introduced β-glucuronidase gene according to Hodal, L. *et al.* (Pl. Sci. (1992), 87: 115-122).

Alternatively, the transgenic genotype of the regenerated shoot may be verified by performing NPTII assays (Radke, S. E. et al, Theor. Appl. Genet. (1988), 75: 685-694) or by performing PCR analysis according to Wang *et al* (1993, NAR 21 pp 4153-4154).

Transfer to soil

The newly rooted plants (height approx. 2-3 cms) are transplanted from rooting substrate to soil and placed in a growth chamber (21°C, 16 hour light 200-400uE/m^2/sec). When the plants are well established they are transferred to the greenhouse, where they are grown until tubers had developed and the upper part of the plants are senescing.

Harvesting

The potatoes are harvested after about 3 months.

TRANSGENIC MAIZE PLANTS

Introduction

Since the first publication of production of transgenic plants in 1983 (Leemans, 1993 Biotechnology **11** s22), there have been numerous publications of production of transgenic plants including especially dicotyledon crop plants.

Until very recently there are very few reports on successful production of transgenic monocotyledononary crop plants. This relatively slow development within monocots are due to two causes. Firstly, until the early 1980s, efficient regeneration of plants from cultured cells and tissues of monocots had proven very difficult. This problem is ultimately solved by the culture of explants from immature and embryogenic tissue, which retain their morphogenic potential on nutrient media containing plant growth regulators. Secondly, the monocots are not a natural host for *Agrobacterium tumefaciens*, meaning that the successful developed techniques within the dicots using their natural vector *Agrobacterium tumefaciens* is unsuccessful for many years in the monocots.

Nevertheless, it is now possible to successfully transformation and produce fertile

transgenic plants of maize using methods such as: (1) Silicon Carbide Whiskers; (2) Particle Bombardment; (3) DNA Uptake by PEG treated protoplast; or (4) DNA Uptake in Electroporation of Tissue. Each of these methods - which are reviewed by Thompson (1995 Euphtytica **85** pp 75-80) - may be used to prepare *inter alia* transgenic maize according to the present invention.

In particular, the particle Gun method has been successfully used for the transformation of monocots. However, EP-A-0604662 reports on a different method of transforming monocotyledons. The method comprises transforming cultured tissues of a monocotyledon under or after dedifferentiation with *Agrobacterium* containing a super binary vector as a selectable marker a hygromycin-resistant gene is used. Production of transgenic calli and plant is demonstrated using the hygromycin selection. This method may be used to prepare *inter alia* transgenic maize according to the present invention.

Subsequent to the method of EP-A-0604662, EP-A-0672752 reports on non-dedifferentiated immature embryos. In this regard, both hygromycin-resistance and PPT-resistance genes are used as the selectable marker, with PPT giving rise to 10% or more independent transformed plants. This method may be used to prepare *inter alia* transgenic maize according to the present invention.

To date, it would appear that transgenic maize plants can be successfully produced from easily-culturable varieties - such as the inbred line A188. In this regard, see the teachings of Ishida *et al* (1996 Nature Biotechnology **14** pp 745-750). The method disclosed by these workers may be used to prepare *inter alia* transgenic maize according to the present invention.

Vasil (1996 Nature Biotechnology **14** pp 702-703) presents a further review article on transformation of maize. Even though it is possible to prepare transformed maize by use of, for example, particle Gun mediated transformation, for the present studies the following protocol is adopted.

Plasmid construction

The disarmed *Agrobacterium tumefaciens* strain LBA 4404, containing the helper *vir* plasmid pRAL4404 (Hoekema *et al*, 1983 Nature **303** pp 179-180), is cultured on YMB agar ($K_2HPO_4.3H_2O$ 660 mg l^{-1}, $MgSO_4$ 200 mg l^{-1}, NaCl 100 mg l^{-1}, mannitol 10 g l^{-1}, yeast extract 400 mg l^{-1}, 0.8% w/v agar, pH 7.0) containing 100 mg l^{-1} rifampicin and 500 mg l^{-1} streptomycin sulphate. Transformation with pVICTOR IV GNG E35S *nagB* IV2' or pVICTOR IV GNG rbc *nagB* IV2' or pVICTOR IV GNG E35S *nagB'* is accomplished using the freeze-thaw method of Holters *et al* (1978 Mol Gen Genet **163** 181-187) and transformants are selected on YMB agar containing 100 mg l^{-1} rifampicin and 500 mg l^{-1} streptomycin, and 50 mg l^{-1} gentamycin sulphate.

Isolation and cocultivation of explants

Immature embryos of, for example, maize line A188 of the size between 1.5 to 2.5 mm are isolated and cocultivated with *Agrobacterium tumefaciens* strain LBA 4404 in N6-AS for 2-3 days at 25°C under illumination. Thereafter, the embryos are washed with sterilized water containing 250 mg/l of cefotaxime and transferred to an LS medium and 250 mg/l cefotaxime and glucosamine in concentrations of up to 100 mg/l (the medium is hereafter called LSS1).

Conditions for the selection of transgenic plants

The explants are cultured for three weeks on LSS1 medium and then transferred to an LS medium containing glucosamine and cefotaxime. After three weeks on this medium, green shoots are isolated.

Rooting of transformed shoots

Transformed shoots are transferred to an MS medium containing 2 mg/l for rooting. After four weeks on this medium, plantlets are transferred to pots with sterile soil for acclimatisation.

TRANSGENIC GUAR PLANTS

Transformation of guar cotyledonary explants is performed according to Joersbo and Okkels (PCT/DK95/00221) using *Agrobacterium tumefaciens* LBA4404 harbouring a suitable plasmid.

Other plants may be transformed in accordance with the present invention, such as other fruits, other vegetables, and other plants such as coffee plants, tea plants etc.

Other modifications of the present invention will be apparent to those skilled in the art.

SEQUENCES

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: DANISCO A/S
(B) STREET: LANGEBROGADE 1
(C) CITY: COPENHAGEN
(D) STATE: COPENHAGEN K
(E) COUNTRY: DENMARK
(F) POSTAL CODE (ZIP): DK-1001

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1088 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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        35                  40                  45
Ser Met Thr Ala Leu Ser Asp Lys Gln Thr Ala Thr Ala Gly Ser Thr
    50                  55                  60
Asp Asn Pro Asp Gly Ile Asp Tyr Lys Thr Tyr Asp Tyr Val Gly Val
65                  70                  75                  80
Trp Gly Phe Ser Pro Leu Ser Asn Thr Asn Trp Phe Ala Ala Gly Ser
                85                  90                  95
Ser Thr Pro Gly Gly Ile Thr Asp Trp Thr Ala Thr Met Asn Val Asn
            100                 105                 110
Phe Asp Arg Ile Asp Asn Pro Ser Ile Thr Val Gln His Pro Val Gln
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Val Gln Val Thr Ser Tyr Asn Asn Asn Ser Tyr Arg Val Arg Phe Asn
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Pro Asp Gly Pro Ile Arg Asp Val Thr Arg Gly Pro Ile Leu Lys Gln
145                 150                 155                 160
Gln Leu Asp Trp Ile Arg Thr Gln Glu Leu Ser Glu Gly Cys Asp Pro
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Gly Met Thr Phe Thr Ser Glu Gly Phe Leu Thr Phe Glu Thr Lys Asp
            180                 185                 190
Leu Ser Val Ile Ile Tyr Gly Asn Phe Lys Thr Arg Val Thr Arg Lys
        195                 200                 205
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Ser Gly Asn Lys Cys Arg Gly Leu Met Phe Val Asp Arg Leu Tyr Gly
225                 230                 235                 240
Asn Ala Ile Ala Ser Val Asn Lys Asn Phe Arg Asn Asp Ala Val Lys
                245                 250                 255
Gln Glu Gly Phe Tyr Gly Ala Gly Glu Val Asn Cys Lys Tyr Gln Asp
            260                 265                 270
Thr Tyr Ile Leu Glu Arg Thr Gly Ile Ala Met Thr Asn Tyr Asn Tyr
        275                 280                 285
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Gly Ala Leu Asn Pro Asp Tyr Tyr Ile Pro Met Tyr Tyr Ala Ala Pro
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595 600 605
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610 615 620
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625 630 635 640
Thr Tyr His Pro Gln Val Leu Val Thr Asp Met Arg Tyr Glu Asn His
645 650 655
Gly Arg Glu Pro Met Val Thr Gln Arg Asn Ile His Ala Tyr Thr Leu
660 665 670
Cys Glu Ser Thr Arg Lys Glu Gly Ile Val Glu Asn Ala Asp Thr Leu
675 680 685
Thr Lys Phe Arg Arg Ser Tyr Ile Ile Ser Arg Gly Gly Tyr Ile Gly
690 695 700
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755 760 765
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770 775 780
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785 790 795 800
Tyr Pro Asn Glu Met Asp Thr Leu Arg Lys Phe Val Glu Phe Arg Tyr
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Arg Trp Gln Glu Val Leu Tyr Thr Ala Met Tyr Gln Asn Ala Ala Phe
820 825 830
Gly Lys Pro Ile Ile Lys Ala Ala Ser Met Tyr Asn Asn Asp Ser Asn
835 840 845
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Tyr Arg Ile Leu Cys Ala Pro Val Val Trp Glu Asn Ser Thr Glu Arg
865 870 875 880

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                885                 890                 895
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            900                 905                 910
Tyr Asn Tyr Pro Val Pro Gln Ser Glu Ser Pro Ile Phe Val Arg Glu
        915                 920                 925
Gly Ala Ile Leu Pro Thr Arg Tyr Thr Leu Asn Gly Glu Asn Lys Ser
    930                 935                 940
Leu Asn Thr Tyr Thr Asp Glu Asp Pro Leu Val Phe Glu Val Phe Pro
945                 950                 955                 960
Leu Gly Asn Asn Arg Ala Asp Gly Met Cys Tyr Leu Asp Asp Gly Gly
                965                 970                 975
Val Thr Thr Asn Ala Glu Asp Asn Gly Lys Phe Ser Val Val Lys Val
            980                 985                 990
Ala Ala Glu Gln Asp Gly Gly Thr Glu Thr Ile Thr Phe Thr Asn Asp
        995                 1000                1005
Cys Tyr Glu Tyr Val Phe Gly Gly Pro Phe Tyr Val Arg Val Arg Gly
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Ala Gln Ser Pro Ser Asn Ile His Val Ser Ser Gly Ala Gly Ser Gln
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Gly Glu Asn Gly Asp Phe Trp Val Asp Gln Glu Thr Asp Ser Leu Trp
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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1091 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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        35                  40                  45
Val Ser Met Ser Ala Leu Phe Asp Lys Pro Thr Ala Val Thr Gly Gly
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Lys Asp Asn Pro Asp Asn Ile Asn Tyr Thr Thr Tyr Asp Tyr Val Pro
65                  70                  75                  80
Val Trp Arg Phe Asp Pro Leu Ser Asn Thr Asn Trp Phe Ala Ala Gly
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Ser Ser Thr Pro Gly Asp Ile Asp Asp Trp Thr Ala Thr Met Asn Val
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Asn Phe Asp Arg Ile Asp Asn Pro Ser Phe Thr Leu Glu Lys Pro Val
        115                 120                 125
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                165                 170                 175
Pro Lys Met Gly Phe Thr Lys Glu Gly Phe Leu Lys Phe Glu Thr Lys
            180                 185                 190
Asp Leu Asn Val Ile Ile Tyr Gly Asn Phe Lys Thr Arg Val Thr Arg
        195                 200                 205
Lys Arg Asp Gly Lys Gly Ile Met Glu Asn Asn Glu Val Pro Ala Gly
    210                 215                 220
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275 280 285
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Leu Ile Ala Pro Gly Tyr Pro Ser Asp Pro Asn Phe Tyr Ile Pro Met
305 310 315 320
Tyr Phe Ala Ala Pro Trp Val Val Val Lys Gly Cys Ser Gly Asn Ser
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340 345 350
Tyr Met Asn Thr Gly Gly Thr Ser Trp Asn Cys Gly Glu Glu Asn Leu
355 360 365
Ala Tyr Met Gly Ala Gln Cys Gly Pro Phe Asp Gln His Phe Val Tyr
370 375 380
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385 390 395 400
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405 410 415
Pro Pro Lys Tyr Val Phe Gly Tyr Phe Gln Gly Val Phe Gly Ile Ala
420 425 430
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435 440 445
Gln Glu Ile Val Glu Gly Tyr Gln Ser Asn Asn Phe Pro Leu Glu Gly
450 455 460
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485 490 495
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500 505 510
Gln Thr Asn Val Thr Cys Phe Leu Arg Asn Asp Asn Gly Gly Ala Asp
515 520 525
Tyr Glu Val Asn Gln Thr Leu Arg Glu Lys Gly Leu Tyr Thr Lys Asn
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595 600 605
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625 630 635 640
Gly Arg Tyr Asn Trp Lys Ser Tyr His Pro Gln Val Leu Val Thr Asp
645 650 655
Met Arg Tyr Glu Asn His Gly Arg Glu Pro Met Phe Thr Gln Arg Asn
660 665 670
Met His Ala Tyr Thr Leu Cys Glu Ser Thr Arg Lys Glu Gly Ile Val
675 680 685
Ala Asn Ala Asp Thr Leu Thr Lys Phe Arg Arg Ser Tyr Ile Ile Ser
690 695 700
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Val Asn Met Asn Met Ser Cys Leu Pro Leu Val Gly Ser Asp Ile Gly
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785                 790                 795                 800
Leu Tyr Met Tyr Lys Asp Glu Met Ala Thr Leu Arg Lys Phe Ile Glu
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        835                 840                 845
Asp Arg Asn Val Arg Gly Ala Gln Asp Asp His Phe Leu Leu Gly Gly
    850                 855                 860
His Asp Gly Tyr Arg Ile Leu Cys Ala Pro Val Val Trp Glu Asn Thr
865                 870                 875                 880
Thr Ser Arg Asp Leu Tyr Leu Pro Val Leu Thr Lys Trp Tyr Lys Phe
                885                 890                 895
Gly Pro Asp Tyr Asp Thr Lys Arg Leu Asp Ser Ala Leu Asp Gly Gly
            900                 905                 910
Gln Met Ile Lys Asn Tyr Ser Val Pro Gln Ser Asp Ser Pro Ile Phe
        915                 920                 925
Val Arg Glu Gly Ala Ile Leu Pro Thr Arg Tyr Thr Leu Asp Gly Ser
    930                 935                 940
Asn Lys Ser Met Asn Thr Tyr Thr Asp Lys Asp Pro Leu Val Phe Glu
945                 950                 955                 960
Val Phe Pro Leu Gly Asn Asn Arg Ala Asp Gly Met Cys Tyr Leu Asp
                965                 970                 975
Asp Gly Gly Ile Thr Thr Asp Ala Glu Asp His Gly Lys Phe Ser Val
            980                 985                 990
Ile Asn Val Glu Ala Leu Arg Lys Gly Val Thr Thr Thr Ile Lys Phe
        995                 1000                1005
Ala Tyr Asp Thr Tyr Gln Tyr Val Phe Asp Gly Pro Phe Tyr Val Arg
    1010                1015                1020
Ile Arg Asn Leu Thr Thr Ala Ser Lys Ile Asn Val Ser Ser Gly Ala
1025                1030                1035                1040
Gly Glu Glu Asp Met Thr Pro Thr Ser Ala Asn Ser Arg Ala Ala Leu
                1045                1050                1055
Phe Ser Asp Gly Gly Val Gly Glu Tyr Trp Ala Asp Asn Asp Thr Ser
            1060                1065                1070
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        1075                1080                1085
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(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1066 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

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        35                  40                  45
Val Asn Leu Arg Phe Asp Asp Gly Thr Leu Gly Val Val Gln Phe Ile
    50                  55                  60
Arg Pro Cys Val Trp Arg Val Arg Tyr Asp Pro Gly Phe Lys Thr Ser
65                  70                  75                  80
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145 150 155 160
Thr Leu Thr Pro Leu Lys Asp Pro Tyr Pro Ile Pro Asn Val Ala Ala
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Ala Glu Ala Arg Val Ser Asp Lys Val Val Trp Gln Thr Ser Pro Lys
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Thr Phe Arg Lys Asn Leu His Pro Gln His Lys Met Leu Lys Asp Thr
195 200 205
Val Leu Asp Ile Val Lys Pro Gly His Gly Glu Tyr Val Gly Trp Gly
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Glu Met Gly Gly Ile Gln Phe Met Lys Glu Pro Thr Phe Met Asn Tyr
225 230 235 240
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245 250 255
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260 265 270
Val Asn Ser Asn Pro Glu His Lys Asn Ile Thr Ala Thr Phe Ile Asp
275 280 285
Asn Tyr Ser Gln Ile Ala Ile Asp Phe Gly Lys Thr Asn Ser Gly Tyr
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325 330 335
Arg Ser Lys Leu Lys Pro Arg Tyr Ile Leu Gly Ala His Gln Ala Cys
340 345 350
Tyr Gly Tyr Gln Gln Glu Ser Asp Leu Tyr Ser Val Val Gln Gln Tyr
355 360 365
Arg Asp Cys Lys Phe Pro Leu Asp Gly Ile His Val Asp Val Asp Val
370 375 380
Gln Asp Gly Phe Arg Thr Phe Thr Thr Asn Pro His Thr Phe Pro Asn
385 390 395 400
Pro Lys Glu Met Phe Thr Asn Leu Arg Asn Asn Gly Ile Lys Cys Ser
405 410 415
Thr Asn Ile Thr Pro Val Ile Ser Ile Asn Asn Arg Glu Gly Gly Tyr
420 425 430
Ser Thr Leu Leu Glu Gly Val Asp Lys Lys Tyr Phe Ile Met Asp Asp
435 440 445
Arg Tyr Thr Glu Gly Thr Ser Gly Asn Ala Lys Asp Val Arg Tyr Met
450 455 460
Tyr Tyr Gly Gly Gly Asn Lys Val Glu Val Asp Pro Asn Asp Val Asn
465 470 475 480
Gly Arg Pro Asp Phe Lys Asp Asn Tyr Asp Phe Pro Ala Asn Phe Asn
485 490 495
Ser Lys Gln Tyr Pro Tyr His Gly Gly Val Ser Tyr Gly Tyr Gly Asn
500 505 510
Gly Ser Ala Gly Phe Tyr Pro Asp Leu Asn Arg Lys Glu Val Arg Ile
515 520 525
Trp Trp Gly Met Gln Tyr Lys Tyr Leu Phe Asp Met Gly Leu Glu Phe
530 535 540
Val Trp Gln Asp Met Thr Thr Pro Ala Ile His Thr Ser Tyr Gly Asp
545 550 555 560
Met Lys Gly Leu Pro Thr Arg Leu Leu Val Thr Ser Asp Ser Val Thr
565 570 575
Asn Ala Ser Glu Lys Lys Leu Ala Ile Glu Thr Trp Ala Leu Tyr Ser
580 585 590
Tyr Asn Leu His Lys Ala Thr Trp His Gly Leu Ser Arg Leu Glu Ser
595 600 605

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Arg Lys Asn Lys Arg Asn Phe Ile Leu Gly Arg Gly Ser Tyr Ala Gly
    610                 615                 620
Ala Tyr Arg Phe Ala Gly Leu Trp Thr Gly Asp Asn Ala Ser Asn Trp
625                 630                 635                 640
Glu Phe Trp Lys Ile Ser Val Ser Gln Val Leu Ser Leu Gly Leu Asn
                645                 650                 655
Gly Val Cys Ile Ala Gly Ser Asp Thr Gly Gly Phe Glu Pro Tyr Arg
            660                 665                 670
Asp Ala Asn Gly Val Glu Glu Lys Tyr Cys Ser Pro Glu Leu Leu Ile
        675                 680                 685
Arg Trp Tyr Thr Gly Ser Phe Leu Leu Pro Trp Leu Arg Asn His Tyr
    690                 695                 700
Val Lys Lys Asp Arg Lys Trp Phe Gln Glu Pro Tyr Ser Tyr Pro Lys
705                 710                 715                 720
His Leu Glu Thr His Pro Glu Leu Ala Asp Gln Ala Trp Leu Tyr Lys
                725                 730                 735
Ser Val Leu Glu Ile Cys Arg Tyr Tyr Val Glu Leu Arg Tyr Ser Leu
            740                 745                 750
Ile Gln Leu Leu Tyr Asp Cys Met Phe Gln Asn Val Val Asp Gly Met
        755                 760                 765
Pro Ile Thr Arg Ser Met Leu Leu Thr Asp Thr Glu Asp Thr Thr Phe
    770                 775                 780
Phe Asn Glu Ser Gln Lys Phe Leu Asp Asn Gln Tyr Met Ala Gly Asp
785                 790                 795                 800
Asp Ile Leu Val Ala Pro Ile Leu His Ser Arg Lys Glu Ile Pro Gly
                805                 810                 815
Glu Asn Arg Asp Val Tyr Leu Pro Leu Tyr His Thr Trp Tyr Pro Ser
            820                 825                 830
Asn Leu Arg Pro Trp Asp Asp Gln Gly Val Ala Leu Gly Asn Pro Val
        835                 840                 845
Glu Gly Gly Ser Val Ile Asn Tyr Thr Ala Arg Ile Val Ala Pro Glu
    850                 855                 860
Asp Tyr Asn Leu Phe His Ser Val Val Pro Val Tyr Val Arg Glu Gly
865                 870                 875                 880
Ala Ile Ile Pro Gln Ile Glu Val Arg Gln Trp Thr Gly Gln Gly Gly
                885                 890                 895
Ala Asn Arg Ile Lys Phe Asn Ile Tyr Pro Gly Lys Asp Lys Glu Tyr
            900                 905                 910
Cys Thr Tyr Leu Asp Asp Gly Val Ser Arg Asp Ser Ala Pro Glu Asp
        915                 920                 925
Leu Pro Gln Tyr Lys Glu Thr His Glu Gln Ser Lys Val Glu Gly Ala
    930                 935                 940
Glu Ile Ala Lys Gln Ile Gly Lys Lys Thr Gly Tyr Asn Ile Ser Gly
945                 950                 955                 960
Thr Asp Pro Glu Ala Lys Gly Tyr His Arg Lys Val Ala Val Thr Gln
                965                 970                 975
Thr Ser Lys Asp Lys Thr Arg Thr Val Thr Ile Glu Pro Lys His Asn
            980                 985                 990
Gly Tyr Asp Pro Ser Lys Glu Val Gly Asp Tyr Tyr Thr Ile Ile Leu
        995                 1000                1005
Trp Tyr Ala Pro Gly Phe Asp Gly Ser Ile Val Asp Val Ser Lys Thr
    1010                1015                1020
Thr Val Asn Val Glu Gly Gly Val Glu His Gln Val Tyr Lys Asn Ser
1025                1030                1035                1040
Asp Leu His Thr Val Val Ile Asp Val Lys Glu Val Ile Gly Thr Thr
                1045                1050                1055
Lys Ser Val Lys Ile Thr Cys Thr Ala Ala
            1060                1065
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1070 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
Met Ala Gly Leu Ser Asp Pro Leu Asn Phe Cys Lys Ala Glu Asp Tyr
1               5                  10                  15
Tyr Ala Ala Ala Lys Gly Trp Ser Gly Pro Gln Lys Ile Ile Arg Tyr
            20                  25                  30
Asp Gln Thr Pro Pro Gln Gly Thr Lys Asp Pro Lys Ser Trp His Ala
        35                  40                  45
Val Asn Leu Pro Phe Asp Asp Gly Thr Met Cys Val Val Gln Phe Val
    50                  55                  60
Arg Pro Cys Val Trp Arg Val Arg Tyr Asp Pro Ser Val Lys Thr Ser
65                  70                  75                  80
Asp Glu Tyr Gly Asp Glu Asn Thr Arg Thr Ile Val Gln Asp Tyr Met
                85                  90                  95
Thr Thr Leu Val Gly Asn Leu Asp Ile Phe Arg Gly Leu Thr Trp Val
            100                 105                 110
Ser Thr Leu Glu Asp Ser Gly Glu Tyr Tyr Thr Phe Lys Ser Glu Val
        115                 120                 125
Thr Ala Val Asp Glu Thr Glu Arg Thr Arg Asn Lys Val Gly Asp Gly
    130                 135                 140
Leu Lys Ile Tyr Leu Trp Lys Asn Pro Phe Arg Ile Gln Val Val Arg
145                 150                 155                 160
Leu Leu Thr Pro Leu Val Asp Pro Phe Pro Ile Pro Asn Val Ala Asn
                165                 170                 175
Ala Thr Ala Arg Val Ala Asp Lys Val Val Trp Gln Thr Ser Pro Lys
            180                 185                 190
Thr Phe Arg Lys Asn Leu His Pro Gln His Lys Met Leu Lys Asp Thr
        195                 200                 205
Val Leu Asp Ile Ile Lys Pro Gly His Gly Glu Tyr Val Gly Trp Gly
    210                 215                 220
Glu Met Gly Gly Ile Glu Phe Met Lys Glu Pro Thr Phe Met Asn Tyr
225                 230                 235                 240
Phe Asn Phe Asp Asn Met Gln Tyr Gln Gln Val Tyr Ala Gln Gly Ala
                245                 250                 255
Leu Asp Ser Arg Glu Pro Leu Tyr His Ser Asp Pro Phe Tyr Leu Asp
            260                 265                 270
Val Asn Ser Asn Pro Glu His Lys Asn Ile Thr Ala Thr Phe Ile Asp
        275                 280                 285
Asn Tyr Ser Gln Ile Ala Ile Asp Phe Gly Lys Thr Asn Ser Gly Tyr
    290                 295                 300
Ile Lys Leu Gly Thr Arg Tyr Gly Gly Ile Asp Cys Tyr Gly Ile Ser
305                 310                 315                 320
Ala Asp Thr Val Pro Glu Ile Val Arg Leu Tyr Thr Gly Leu Val Gly
                325                 330                 335
Arg Ser Lys Leu Lys Pro Arg Tyr Ile Leu Gly Ala His Gln Ala Cys
            340                 345                 350
Tyr Gly Tyr Gln Gln Glu Ser Asp Leu His Ala Val Val Gln Gln Tyr
        355                 360                 365
Arg Asp Thr Lys Phe Pro Leu Asp Gly Leu His Val Asp Val Asp Phe
    370                 375                 380
Gln Asp Asn Phe Arg Thr Phe Thr Thr Asn Pro Ile Thr Phe Pro Asn
385                 390                 395                 400
Pro Lys Glu Met Phe Thr Asn Leu Arg Asn Asn Gly Ile Lys Cys Ser
                405                 410                 415
Thr Asn Ile Thr Pro Val Ile Ser Ile Arg Asp Arg Pro Asn Gly Tyr
            420                 425                 430
Ser Thr Leu Asn Glu Gly Tyr Asp Lys Lys Tyr Phe Ile Met Asp Asp
        435                 440                 445
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Arg Tyr Thr Glu Gly Thr Ser Gly Asp Pro Gln Asn Val Arg Tyr Ser
450 455 460
Phe Tyr Gly Gly Gly Asn Pro Val Glu Val Asn Pro Asn Asp Val Trp
465 470 475 480
Ala Arg Pro Asp Phe Gly Asp Asn Tyr Asp Phe Pro Thr Asn Phe Asn
485 490 495
Cys Lys Asp Tyr Pro Tyr His Gly Gly Val Ser Tyr Gly Tyr Gly Asn
500 505 510
Gly Thr Pro Gly Tyr Tyr Pro Asp Leu Asn Arg Glu Glu Val Arg Ile
515 520 525
Trp Trp Gly Leu Gln Tyr Glu Tyr Leu Phe Asn Met Gly Leu Glu Phe
530 535 540
Val Trp Gln Asp Met Thr Thr Pro Ala Ile His Ser Ser Tyr Gly Asp
545 550 555 560
Met Lys Gly Leu Pro Thr Arg Leu Leu Val Thr Ala Asp Ser Val Thr
565 570 575
Asn Ala Ser Glu Lys Lys Leu Ala Ile Glu Ser Trp Ala Leu Tyr Ser
580 585 590
Tyr Asn Leu His Lys Ala Thr Phe His Gly Leu Gly Arg Leu Glu Ser
595 600 605
Arg Lys Asn Lys Arg Asn Phe Ile Leu Gly Arg Gly Ser Tyr Ala Gly
610 615 620
Ala Tyr Arg Phe Ala Gly Leu Trp Thr Gly Asp Asn Ala Ser Thr Trp
625 630 635 640
Glu Phe Trp Lys Ile Ser Val Ser Gln Val Leu Ser Leu Gly Leu Asn
645 650 655
Gly Val Cys Ile Ala Gly Ser Asp Thr Gly Gly Phe Glu Pro Ala Arg
660 665 670
Thr Glu Ile Gly Glu Glu Lys Tyr Cys Ser Pro Glu Leu Leu Ile Arg
675 680 685
Trp Tyr Thr Gly Ser Phe Leu Leu Pro Trp Leu Arg Asn His Tyr Val
690 695 700
Lys Lys Asp Arg Lys Trp Phe Gln Glu Pro Tyr Ala Tyr Pro Lys His
705 710 715 720
Leu Glu Thr His Pro Glu Leu Ala Asp Gln Ala Trp Leu Tyr Lys Ser
725 730 735
Val Leu Glu Ile Cys Arg Tyr Trp Val Glu Leu Arg Tyr Ser Leu Ile
740 745 750
Gln Leu Leu Tyr Asp Cys Met Phe Gln Asn Val Val Asp Gly Met Pro
755 760 765
Leu Ala Arg Ser Met Leu Leu Thr Asp Thr Glu Asp Thr Thr Phe Phe
770 775 780
Asn Glu Ser Gln Lys Phe Leu Asp Asn Gln Tyr Met Ala Gly Asp Asp
785 790 795 800
Ile Leu Val Ala Pro Ile Leu His Ser Arg Asn Glu Val Pro Gly Glu
805 810 815
Asn Arg Asp Val Tyr Leu Pro Leu Phe His Thr Trp Tyr Pro Ser Asn
820 825 830
Leu Arg Pro Trp Asp Asp Gln Gly Val Ala Leu Gly Asn Pro Val Glu
835 840 845
Gly Gly Ser Val Ile Asn Tyr Thr Ala Arg Ile Val Ala Pro Glu Asp
850 855 860
Tyr Asn Leu Phe His Asn Val Val Pro Val Tyr Ile Arg Glu Gly Ala
865 870 875 880
Ile Ile Pro Gln Ile Gln Val Arg Gln Trp Ile Gly Glu Gly Gly Pro
885 890 895
Asn Pro Ile Lys Phe Asn Ile Tyr Pro Gly Lys Asp Lys Glu Tyr Val
900 905 910
Thr Tyr Leu Asp Asp Gly Val Ser Arg Asp Ser Ala Pro Asp Asp Leu
915 920 925
Pro Gln Tyr Arg Glu Ala Tyr Glu Gln Ala Lys Val Glu Gly Lys Asp
930 935 940
Val Gln Lys Gln Leu Ala Val Ile Gln Gly Asn Lys Thr Asn Asp Phe
945 950 955 960
Ser Ala Ser Gly Ile Asp Lys Glu Ala Lys Gly Tyr His Arg Lys Val
965 970 975

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Ser Ile Lys Gln Glu Ser Lys Asp Lys Thr Arg Thr Val Thr Ile Glu
            980                 985                 990
Pro Lys His Asn Gly Tyr Asp Pro Ser Lys Glu Val Gly Asn Tyr Tyr
        995                 1000                1005
Thr Ile Ile Leu Trp Tyr Ala Pro Gly Phe Asp Gly Ser Ile Val Asp
    1010                1015                1020
Val Ser Gln Ala Thr Val Asn Ile Glu Gly Gly Val Glu Cys Glu Ile
1025                1030                1035                1040
Phe Lys Asn Thr Gly Leu His Thr Val Val Val Asn Val Lys Glu Val
                1045                1050                1055
Ile Gly Thr Thr Lys Ser Val Lys Ile Thr Cys Thr Thr Ala
            1060                1065                1070
```

SEQ. ID. NO. 5

SEQUENCE TYPE: ENZYME
MOLECULE TYPE: AMINO ACID
ORIGINAL SOURCE: ALGAL
SEQUENCE LENGTH: 1092 AMINO ACIDS
SEQUENCE:

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                        5                  10                  15
                        |                  |                   |
  1 Met Phe Pro Thr Leu Thr Phe Ile Ala Pro Ser Ala Leu Ala Ala
 16 Ser Thr Phe Val Gly Ala Asp Ile Arg Ser Gly Ile Arg Ile Gln
 31 Ser Ala Leu Pro Ala Val Arg Asn Ala Val Arg Arg Ser Lys His
 46 Tyr Asn Val Ser Met Thr Ala Leu Ser Asp Lys Gln Thr Ala Ile
 61 Ser Ile Gly Pro Asp Asn Pro Asp Gly Ile Asn Tyr Gln Asn Tyr
 76 Asp Tyr Ile Pro Val Ala Gly Phe Thr Pro Leu Ser Asn Thr Asn
 91 Trp Tyr Ala Ala Gly Ser Ser Thr Pro Gly Gly Ile Thr Asp Trp
106 Thr Ala Thr Met Asn Val Lys Phe Asp Arg Ile Asp Asn Pro Ser
121 Tyr Ser Asn Asn His Pro Val Gln Ile Gln Val Thr Ser Tyr Asn
136 Asn Asn Ser Phe Arg Ile Arg Phe Asn Pro Asp Gly Pro Ile Arg
151 Asp Val Ser Arg Gly Pro Ile Leu Lys Gln Gln Leu Thr Trp Ile
166 Arg Asn Gln Glu Leu Ala Gln Gly Cys Asn Pro Asn Met Ser Phe
181 Ser Pro Glu Gly Phe Leu Ser Phe Glu Thr Lys Asp Leu Asn Val
196 Ile Ile Tyr Gly Asn Cys Lys Met Arg Val Thr Lys Lys Asp Gly
211 Tyr Leu Val Met Glu Asn Asp Glu Cys Asn Ser Gln Ser Asp Gly
226 Asn Lys Cys Arg Gly Leu Met Tyr Val Asp Arg Leu Tyr Gly Asn
241 Ala Ile Ala Ser Val Gln Thr Asn Phe His Lys Asp Thr Ser Arg
256 Asn Glu Lys Phe Tyr Gly Ala Gly Glu Val Asn Cys Arg Tyr Glu
271 Glu Gln Gly Lys Ala Pro Thr Tyr Val Leu Glu Arg Ser Gly Leu
286 Ala Met Thr Asn Tyr Asn Tyr Asp Asn Leu Asn Tyr Asn Gln Pro
301 Asp Val Val Pro Pro Gly Tyr Pro Asp His Pro Asn Tyr Tyr Ile
316 Pro Met Tyr Tyr Ala Ala Pro Trp Leu Val Val Gln Gly Cys Ala
331 Gly Thr Ser Lys Gln Tyr Ser Tyr Gly Trp Phe Met Asp Asn Val
346 Ser Gln Ser Tyr Met Asn Thr Gly Asp Thr Ala Trp Asn Cys Gly
361 Gln Glu Asn Leu Ala Tyr Met Gly Ala Gln Tyr Gly Pro Phe Asp
376 Gln His Phe Val Tyr Gly Asp Gly Asp Gly Leu Glu Asp Val Val
391 Lys Ala Phe Ser Phe Leu Gln Gly Lys Glu Phe Glu Asp Lys Lys
406 Leu Asn Lys Arg Ser Val Met Pro Pro Lys Tyr Val Phe Gly Phe
421 Phe Gln Gly Val Phe Gly Ala Leu Ser Leu Leu Lys Gln Asn Leu
436 Pro Ala Gly Glu Asn Asn Ile Ser Val Gln Glu Ile Val Glu Gly
451 Tyr Gln Asp Asn Asp Tyr Pro Phe Glu Gly Leu Ala Val Asp Val
466 Asp Met Gln Asp Asp Leu Arg Val Phe Thr Thr Lys Pro Glu Tyr
481 Trp Ser Ala Asn Met Val Gly Glu Gly Gly Asp Pro Asn Asn Arg
496 Ser Val Phe Glu Trp Ala His Asp Arg Gly Leu Val Cys Gln Thr
511 Asn Val Thr Cys Phe Leu Arg Asn Asp Asn Ser Gly Lys Pro Tyr
526 Glu Val Asn Gln Thr Leu Arg Glu Lys Gln Leu Tyr Thr Lys Asn
541 Asp Ser Leu Asn Asn Thr Asp Phe Gly Thr Thr Ser Asp Gly Pro
556 Gly Asp Ala Tyr Ile Gly His Leu Asp Tyr Gly Gly Gly Val Glu
571 Cys Asp Ala Ile Phe Pro Asp Trp Gly Arg Pro Asp Val Ala Gln
586 Trp Trp Gly Glu Asn Tyr Lys Lys Leu Phe Ser Ile Gly Leu Asp
601 Phe Val Trp Gln Asp Met Thr Val Pro Ala Met Met Pro His Arg
616 Leu Gly Asp Ala Val Asn Lys Asn Ser Gly Ser Ser Ala Pro Gly
631 Trp Pro Asn Glu Asn Asp Pro Ser Asn Gly Arg Tyr Asn Trp Lys
646 Ser Tyr His Pro Gln Val Leu Val Thr Asp Met Arg Tyr Gly Ala
661 Glu Tyr Gly Arg Glu Pro Met Val Ser Gln Arg Asn Ile His Ala
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 676 Tyr Thr Leu Cys Glu Ser Thr Arg Arg Glu Gly Ile Val Gly Asn
 691 Ala Asp Ser Leu Thr Lys Phe Arg Arg Ser Tyr Ile Ile Ser Arg
 706 Gly Gly Tyr Ile Gly Asn Gln His Phe Gly Gly Met Trp Val Gly
 721 Asp Asn Ser Ala Thr Glu Ser Tyr Leu Gln Met Met Leu Ala Asn
 736 Ile Ile Asn Met Asn Met Ser Cys Leu Pro Leu Val Gly Ser Asp
 751 Ile Gly Gly Phe Thr Gln Tyr Asn Asp Ala Gly Asp Pro Thr Pro
 766 Glu Asp Leu Met Val Arg Phe Val Gln Ala Gly Cys Leu Leu Pro
 781 Trp Phe Arg Asn His Tyr Asp Arg Trp Ile Glu Ser Lys Lys His
 796 Gly Lys Lys Tyr Gln Glu Leu Tyr Met Tyr Pro Gly Gln Lys Asp
 811 Thr Leu Lys Lys Phe Val Glu Phe Arg Tyr Arg Trp Gln Glu Val
 826 Leu Tyr Thr Ala Met Tyr Gln Asn Ala Thr Thr Gly Glu Pro Ile
 841 Ile Lys Ala Ala Pro Met Tyr Asn Asn Asp Val Asn Val Tyr Lys
 856 Ser Gln Asn Asp His Phe Leu Leu Gly Gly His Asp Gly Tyr Arg
 871 Ile Leu Cys Ala Pro Val Val Arg Glu Asn Ala Thr Ser Arg Glu
 886 Val Tyr Leu Pro Val Tyr Ser Lys Trp Phe Lys Phe Gly Pro Asp
 901 Phe Asp Thr Lys Pro Leu Glu Asn Glu Ile Gln Gly Gly Gln Thr
 916 Leu Tyr Asn Tyr Ala Ala Pro Leu Asn Asp Ser Pro Ile Phe Val
 931 Arg Glu Gly Thr Ile Leu Pro Thr Arg Tyr Thr Leu Asp Gly Val
 946 Asn Lys Ser Ile Asn Thr Tyr Thr Asp Asn Asp Pro Leu Val Phe
 961 Glu Leu Phe Pro Leu Glu Asn Asn Gln Ala His Gly Leu Phe Tyr
 976 His Asp Asp Gly Gly Val Thr Thr Asn Ala Glu Asp Phe Gly Lys
 991 Tyr Ser Val Ile Ser Val Lys Ala Ala Gln Glu Gly Ser Gln Met
1006 Ser Val Lys Phe Asp Asn Glu Val Tyr Glu His Gln Trp Gly Ala
1021 Ser Phe Tyr Val Arg Val Arg Asn Met Gly Ala Pro Ser Asn Ile
1036 Asn Val Ser Ser Gln Ile Gly Gln Gln Asp Met Gln Gln Ser Ser
1051 Val Ser Ser Arg Ala Gln Met Phe Thr Ser Ala Asn Asp Gly Glu
1066 Tyr Trp Val Asp Gln Ser Thr Asn Ser Leu Trp Leu Lys Leu Pro
1081 Gly Ala Val Ile Gln Asp Ala Ala Ile Thr Val Arg
```

Number of amino acid residues: 1092

Amino acid composition (including the signal sequense):

64 Ala	14 Cys	18 His	33 Met	56 Thr
48 Arg	55 Gln	45 Ile	49 Phe	22 Trp
89 Asn	49 Glu	65 Leu	59 Pro	67 Tyr
73 Asp	94 Gly	46 Lys	73 Ser	73 Val

SEQ. ID. NO. 6

SEQUENCE TYPE: ENZYME
MOLECULE TYPE: AMINO ACID
ORIGINAL SOURCE: ALGAL
SEQUENCE LENGTH: 570 AMINO ACIDS
SEQUENCE:

```
                      5                  10                  15
                      |                  |                   |
   1 Met Thr Asn Tyr Asn Tyr Asp Asn Leu Asn Tyr Asn Gln Pro Asp
  16 Leu Ile Pro Pro Gly His Asp Ser Asp Pro Asp Tyr Tyr Ile Pro
  31 Met Tyr Phe Ala Ala Pro Trp Val Ile Ala His Gly Tyr Arg Gly
  46 Thr Ser Asp Gln Tyr Ser Tyr Gly Trp Phe Leu Asp Asn Val Ser
  61 Gln Ser Tyr Thr Asn Thr Gly Asp Asp Ala Trp Ala Gly Gln Lys
  76 Asp Leu Ala Tyr Met Gly Ala Gln Cys Gly Pro Phe Asp Gln His
  91 Phe Val Tyr Glu Ala Gly Asp Gly Leu Glu Asp Val Val Thr Ala
 106 Phe Ser Tyr Leu Gln Gly Lys Glu Tyr Glu Asn Gln Gly Leu Asn
 121 Ile Arg Ser Ala Met Pro Pro Lys Tyr Val Phe Gly Phe Phe Gln
 136 Gly Val Phe Gly Ala Thr Ser Leu Leu Arg Asp Asn Leu Pro Ala
 151 Gly Glu Asn Asn Val Ser Leu Glu Glu Ile Val Glu Gly Tyr Gln
 166 Asn Gln Asn Val Pro Phe Glu Gly Leu Ala Val Asp Val Asp Met
 181 Gln Asp Asp Leu Arg Val Phe Thr Thr Arg Pro Ala Phe Trp Thr
 196 Ala Asn Lys Val Gly Glu Gly Gly Asp Pro Asn Asn Lys Ser Val
 211 Phe Glu Trp Ala His Asp Arg Gly Leu Val Cys Gln Thr Asn Val
 226 Thr Cys Phe Leu Lys Asn Glu Lys Asn Pro Tyr Glu Val Asn Gln
 241 Ser Leu Arg Glu Lys Gln Leu Tyr Thr Lys Ser Asp Ser Leu Asp
 256 Asn Ile Asp Phe Gly Thr Thr Pro Asp Gly Pro Ser Asp Ala Tyr
 271 Ile Gly His Leu Asp Tyr Gly Gly Gly Val Glu Cys Asp Ala Leu
 286 Phe Pro Asp Trp Gly Arg Pro Asp Val Ala Gln Trp Trp Gly Asp
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301 Asn Tyr Lys Lys Leu Phe Ser Ile Gly Leu Asp Phe Val Trp Gln
316 Asp Met Thr Val Pro Ala Met Met Pro His Arg Leu Gly Asp Pro
331 Val Gly Thr Asn Ser Gly Glu Thr Ala Pro Gly Trp Pro Asn Asp
346 Lys Asp Pro Ser Asn Gly Arg Tyr Asn Trp Lys Ser Tyr His Pro
361 Gln Val Leu Val Thr Asp Met Arg Tyr Asp Asp Tyr Gly Arg Asp
376 Pro Ile Val Thr Gln Arg Asn Leu His Ala Tyr Thr Leu Cys Glu
391 Ser Thr Arg Arg Glu Gly Ile Val Gly Asn Ala Asp Ser Leu Thr
406 Lys Phe Arg Arg Ser Tyr Ile Ile Ser Arg Gly Gly Tyr Ile Gly
421 Asn Gln His Phe Gly Gly Met Trp Val Gly Asp Asn Ser Ser Thr
436 Glu Asp Tyr Leu Ala Met Met Val Ile Asn Val Ile Asn Met Asn
451 Met Ser Gly Val Pro Leu Val Gly Ser Asp Ile Gly Gly Phe Thr
466 Glu His Asp Lys Arg Asn Pro Cys Thr Pro Asp Leu Met Met Arg
481 Phe Val Gln Ala Gly Cys Leu Leu Pro Trp Phe Arg Asn His Tyr
496 Asp Arg Trp Ile Glu Ser Lys Lys His Gly Lys Asn Tyr Gln Glu
511 Leu Tyr Met Tyr Arg Asp His Leu Asp Ala Leu Arg Ser Phe Val
526 Glu Leu Arg Tyr Arg Trp Gln Glu Val Leu Tyr Thr Ala Met Tyr
541 Gln Asn Ala Leu Asn Gly Lys Pro Ile Ile Lys Thr Val Ser Met
556 Tyr Asn Asn Asp Met Asn Val Lys Asp Ala Gln Asn Asp His Phe

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3267 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

ATGTTTTCAA CCCTTGCGTT TGTCGCACCT AGTGCGCTGG GAGCCAGTAC CTTCGTAGGG 60
GCGGAGGTCA GGTCAAATGT TCGTATCCAT TCCGCTTTTC CAGCTGTGCA CACAGCTACT 120
CGCAAAACCA ATCGCCTCAA TGTATCCATG ACCGCATTGT CCGACAAACA AACGGCTACT 180
GCGGGTAGTA CAGACAATCC GGACGGTATC GACTACAAGA CCTACGATTA CGTCGGAGTA 240
TGGGGTTTCA GCCCCCTCTC CAACACGAAC TGGTTTGCTG CCGGCTCTTC TACCCCGGGT 300
GGCATCACTG ATTGGACGGC TACAATGAAT GTCAACTTCG ACCGTATCGA CAATCCGTCC 360
ATCACTGTCC AGCATCCCGT TCAGGTTCAG GTCACGTCAT ACAACAACAA CAGCTACAGG 420
GTTCGCTTCA ACCCTGATGG CCCTATTCGT GATGTGACTC GTGGGCCTAT CCTCAAGCAG 480
CAACTAGATT GGATTCGAAC GCAGGAGCTG TCAGAGGGAT GTGATCCCGG AATGACTTTC 540
ACATCAGAAG GTTTCTTGAC TTTTGAGACC AAGGATCTAA GCGTCATCAT CTACGGAAAT 600
TTCAAGACCA GAGTTACGAG AAAGTCTGAC GGCAAGGTCA TCATGGAAAA TGATGAAGTT 660
GGAACTGCAT CGTCCGGGAA CAAGTGCCGG GGATTGATGT TCGTTGATAG ATTATACGGT 720
AACGCTATCG CTTCCGTCAA CAAGAACTTC CGCAACGACG CGGTCAAGCA GGAGGGATTC 780
TATGGTGCAG GTGAAGTCAA CTGTAAGTAC CAGGACACCT ACATCTTAGA ACGCACTGGA 840
ATCGCCATGA CAAATTACAA CTACGATAAC TTGAACTATA ACCAGTGGGA CCTTAGACCT 900
CCGCATCATG ATGGTGCCCT CAACCCAGAC TATTATATTC CAATGTACTA CGCAGCACCT 960
TGGTTGATCG TTAATGGATG CGCCGGTACT TCGGAGCAGT ACTCGTATGG ATGGTTCATG 1020
GACAATGTCT CTCAATCTTA CATGAATACT GGAGATACTA CCTGGAATTC TGGACAAGAG 1080
GACCTGGCAT ACATGGGCGC GCAGTATGGA CCATTTGACC AACATTTTGT TTACGGTGCT 1140
GGGGGTGGGA TGGAATGTGT GGTCACAGCG TTCTCTCTTC TACAAGGCAA GGAGTTCGAG 1200
AACCAAGTTC TCAACAAACG TTCAGTAATG CCTCCGAAAT ACGTCTTTGG TTTCTTCCAG 1260
GGTGTTTTCG GGACTTCTTC CTTGTTGAGA GCGCATATGC CAGCAGGTGA GAACAACATC 1320
TCAGTCGAAG AAATTGTAGA AGGTTATCAA AACAACAATT TCCCTTTCGA GGGGCTCGCT 1380
GTGGACGTGG ATATGCAAGA CAACTTGCGG GTGTTCACCA CGAAGGGCGA ATTTTGGACC 1440
GCAAACAGGG TGGGTACTGG CGGGGATCCA AACAACCGAT CGGTTTTTGA ATGGGCACAT 1500
GACAAAGGCC TTGTTTGTCA GACAAATATA ACTTGCTTCC TGAGGAATGA TAACGAGGGG 1560
CAAGACTACG AGGTCAATCA GACGTTAAGG GAGAGGCAGT TGTACACGAA GAACGACTCC 1620
CTGACGGGTA CGGATTTTGG AATGACCGAC GACGGCCCCA GCGATGCGTA CATCGGTCAT 1680
CTGGACTATG GGGGTGGAGT AGAATGTGAT GCACTTTTCC CAGACTGGGG ACGGCCTGAC 1740
GTGGCCGAAT GGTGGGGAAA TAACTATAAG AAACTGTTCA GCATTGGTCT CGACTTCGTC 1800
TGGCAAGACA TGACTGTTCC AGCAATGATG CCGCACAAAA TTGGCGATGA CATCAATGTG 1860
AAACCGGATG GGAATTGGCC GAATGCGGAC GATCCGTCCA ATGGACAATA CAACTGGAAG 1920
ACGTACCATC CCCAAGTGCT TGTAACTGAT ATGCGTTATG AGAATCATGG TCGGGAACCG 1980
ATGGTCACTC AACGCAACAT TCATGCGTAT ACACTGTGCG AGTCTACTAG GAAGGAAGGG 2040
ATCGTGGAAA ACGCAGACAC TCTAACGAAG TTCCGCCGTA GCTACATTAT CAGTCGTGGT 2100
GGTTACATTG GTAACCAGCA TTTCGGGGGT ATGTGGGTGG GAGACAACTC TACTACATCA 2160

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AACTACATCC AAATGATGAT TGCCAACAAT ATTAACATGA ATATGTCTTG CTTGCCTCTC    2220
GTCGGCTCCG ACATTGGAGG ATTCACCTCA TACGACAATG AGAATCAGCG AACGCCGTGT    2280
ACCGGGGACT TGATGGTGAG GTATGTGCAG GCGGGCTGCC TGTTGCCGTG GTTCAGGAAC    2340
CACTATGATA GGTGGATCGA GTCCAAGGAC CACGGAAAGG ACTACCAGGA GCTGTACATG    2400
TATCCGAATG AAATGGATAC GTTGAGGAAG TTCGTTGAAT TCCGTTATCG CTGGCAGGAA    2460
GTGTTGTACA CGGCCATGTA CCAGAATGCG GCTTTCGGAA AGCCGATTAT CAAGGCTGCT    2520
TCGATGTACA ATAACGACTC AAACGTTCGC AGGGCGCAGA ACGATCATTT CCTTCTTGGT    2580
GGACATGATG GATATCGCAT TCTGTGCGCG CCTGTTGTGT GGGAGAATTC GACCGAACGC    2640
GAATTGTACT TGCCCGTGCT GACCCAATGG TACAAATTCG GTCCCGACTT TGACACCAAG    2700
CCTCTGGAAG GAGCGATGAA CGGAGGGGAC CGAATTTACA ACTACCCTGT ACCGCAAAGT    2760
GAATCACCAA TCTTCGTGAG AGAAGGTGCG ATTCTCCCTA CCCGCTACAC GTTGAACGGT    2820
GAAAACAAAT CATTGAACAC GTACACGGAC GAAGATCCGT TGGTGTTTGA AGTATTCCCC    2880
CTCGGAAACA ACCGTGCCGA CGGTATGTGT TATCTTGATG ATGGCGGTGT GACCACCAAT    2940
GCTGAAGACA ATGGCAAGTT CTCTGTCGTC AAGGTGGCAG CGGAGCAGGA TGGTGGTACG    3000
GAGACGATAA CGTTTACGAA TGATTGCTAT GAGTACGTTT TCGGTGGACC GTTCTACGTT    3060
CGAGTGCGCG GCGCTCAGTC GCCGTCGAAC ATCCACGTGT CTTCTGGAGC GGGTTCTCAG    3120
GACATGAAGG TGAGCTCTGC CACTTCCAGG GCTGCGCTGT TCAATGACGG GGAGAACGGT    3180
GATTTCTGGG TTGACCAGGA GACAGATTCT CTGTGGCTGA AGTTGCCCAA CGTTGTTCTC    3240
CCGGACGCTG TGATCACAAT TACCTAA                                        3267
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(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3276 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

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ATGTATCCAA CCCTCACCTT CGTGGCGCCT AGTGCGCTAG GGGCCAGAAC TTTCACGTGT      60
GTGGGCATTT TTAGGTCACA CATTCTTATT CATTCGGTTG TTCCAGCGGT GCGTCTAGCT     120
GTGCGCAAAA GCAACCGCCT CAATGTATCC ATGTCCGCTT TGTTCGACAA ACCGACTGCT     180
GTTACTGGAG GGAAGGACAA CCCGGACAAT ATCAATTACA CCACTTATGA CTACGTCCCT     240
GTGTGGCGCT TCGACCCCCT CAGCAATACG AACTGGTTTG CTGCCGGATC TTCCACTCCC     300
GGCGATATTG ACGACTGGAC GGCGACAATG AATGTGAACT TCGACCGTAT CGACAATCCA     360
TCCTTCACTC TCGAGAAACC GGTTCAGGTT CAGGTCACGT CATACAAGAA CAATTGTTTC     420
AGGGTTCGCT TCAACCCTGA TGGTCCTATT CGCGATGTGG ATCGTGGGCC TATCCTCCAG     480
CAGCAACTAA ATTGGATCCG GAAGCAGGAG CAGTCGAAGG GGTTTGATCC TAAGATGGGC     540
TTCACAAAAG AAGGTTTCTT GAAATTTGAG ACCAAGGATC TGAACGTTAT CATATATGGC     600
AATTTTAAGA CTAGAGTTAC GAGGAAGAGG GATGGAAAAG GGATCATGGA GAATAATGAA     660
GTGCCGGCAG GATCGTTAGG GAACAAGTGC CGGGGATTGA TGTTTGTCGA CAGGTTGTAC     720
GGCACTGCCA TCGCTTCCGT TAATGAAAAT TACCGCAACG ATCCCGACAG GAAAGAGGGG     780
TTCTATGGTG CAGGAGAAGT AAACTGCGAG TTTTGGGACT CCGAACAAAA CAGGAACAAG     840
TACATCTTAG AACGAACTGG AATCGCCATG ACAAATTACA ATTATGACAA CTATAACTAC     900
AACCAGTCAG ATCTTATTGC TCCAGGATAT CCTTCCGACC CGAACTTCTA CATTCCCATG     960
TATTTTGCAG CACCTTGGGT AGTTGTTAAG GGATGCAGTG GCAACAGCGA TGAACAGTAC    1020
TCGTACGGAT GGTTTATGGA TAATGTCTCC CAAACTTACA TGAATACTGG TGGTACTTCC    1080
TGGAACTGTG GAGAGGAGAA CTTGGCATAC ATGGGAGCAC AGTGCGGTCC ATTTGACCAA    1140
CATTTTGTGT ATGGTGATGG AGATGGTCTT GAGGATGTTG TCCAAGCGTT CTCTCTTCTG    1200
CAAGGCAAAG AGTTTGAGAA CCAAGTTCTG AACAAACGTG CCGTAATGCC TCCGAAATAT    1260
GTGTTTGGTT ACTTTCAGGG AGTCTTTGGG ATTGCTTCCT TGTTGAGAGA GCAAAGACCA    1320
GAGGGTGGTA ATAACATCTC TGTTCAAGAG ATTGTCGAAG GTTACCAAAG CAATAACTTC    1380
CCTTTAGAGG GGTTAGCCGT AGATGTGGAT ATGCAACAAG ATTTGCGCGT GTTCACCACG    1440
AAGATTGAAT TTTGGACGGC AAATAAGGTA GGCACCGGGG GAGACTCGAA TAACAAGTCG    1500
GTGTTTGAAT GGGCACATGA CAAAGGCCTT GTATGTCAGA CGAATGTTAC TTGCTTCTTG    1560
AGAAACGACA ACGGCGGGGC AGATTACGAA GTCAATCAGA CATTGAGGGA GAAGGGTTTG    1620
TACACGAAGA ATGACTCACT GACGAACACT AACTTCGGAA CTACCAACGA CGGGCCGAGC    1680
GATGCGTACA TTGGACATCT GGACTATGGT GGCGGAGGGA ATTGTGATGC ACTTTTCCCA    1740
GACTGGGGTC GACCGGGTGT GGCTGAATGG TGGGGTGATA ACTACAGCAA GCTCTTCAAA    1800
ATTGGTCTGG ATTTCGTCTG GCAAGACATG ACAGTTCCAG CTATGATGCC ACACAAAGTT    1860
GGCGACGCAG TCGATACGAG ATCACCTTAC GGCTGGCCGA ATGAGAATGA TCCTTCGAAC    1920
GGACGATACA ATTGGAAATC TTACCATCCA CAAGTTCTCG TAACTGATAT GCGATATGAG    1980
AATCATGGAA GGGAACCGAT GTTCACTCAA CGCAATATGC ATGCGTACAC ACTCTGTGAA    2040
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TCTACGAGGA AGGAAGGGAT TGTTGCAAAT GCAGACACTC TAACGAAGTT CCGCCGCAGT    2100
TATATTATCA GTCGTGGAGG TTACATTGGC AACCAGCATT TTGGAGGAAT GTGGGTTGGA    2160
GACAACTCTT CCTCCCAAAG ATACCTCCAA ATGATGATCG CGAACATCGT CAACATGAAC    2220
ATGTCTTGCC TTCCACTAGT TGGGTCCGAC ATTGGAGGTT TTACTTCGTA TGATGGACGA    2280
AACGTGTGTC CCGGGGATCT AATGGTAAGA TTCGTGCAGG CGGGTTGCTT ACTACCGTGG    2340
TTCAGAAACC ACTATGGTAG GTTGGTCGAG GGCAAGCAAG AGGGAAAATA CTATCAAGAA    2400
CTGTACATGT ACAAGGACGA GATGGCTACA TTGAGAAAAT TCATTGAATT CCGTTACCGC    2460
TGGCAGGAGG TGTTGTACAC TGCTATGTAC CAGAATGCGG CTTTCGGGAA ACCGATTATC    2520
AAGGCAGCTT CCATGTACGA CAACGACAGA AACGTTCGCG GCGCACAGGA TGACCACTTC    2580
CTTCTCGGCG GACACGATGG ATATCGTATT TTGTGTGCAC CTGTTGTGTG GGAGAATACA    2640
ACCAGTCGCG ATCTGTACTT GCCTGTGCTG ACCAAATGGT ACAAATTCGG CCCTGACTAT    2700
GACACCAAGC GCCTGGATTC TGCGTTGGAT GGAGGGCAGA TGATTAAGAA CTATTCTGTG    2760
CCACAAAGCG ACTCTCCGAT ATTTGTGAGG GAAGGAGCTA TTCTCCCTAC CCGCTACACG    2820
TTGGACGGTT CGAACAAGTC AATGAACACG TACACAGACA AAGACCCGTT GGTGTTTGAG    2880
GTATTCCCTC TTGGAAACAA CCGTGCCGAC GGTATGTGTT ATCTTGATGA TGGCGGTATT    2940
ACTACAGATG CTGAGGACCA TGGCAAATTC TCTGTTATCA ATGTCGAAGC CTTACGGAAA    3000
GGTGTTACGA CGACGATCAA GTTTGCGTAT GACACTTATC AATACGTATT TGATGGTCCA    3060
TTCTACGTTC GAATCCGTAA TCTTACGACT GCATCAAAAA TTAACGTGTC TTCTGGAGCG    3120
GGTGAAGAGG ACATGACACC GACCTCTGCG AACTCGAGGG CAGCTTTGTT CAGTGATGGA    3180
GGTGTTGGAG AATACTGGGC TGACAATGAT ACGTCTTCTC TGTGGATGAA GTTGCCAAAC    3240
CTGGTTCTGC AAGACGCTGT GATTACCATT ACGTAG                               3276
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(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3201 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

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ATGGCAGGAT TTTCTGATCC TCTCAACTTT TGCAAAGCAG AAGACTACTA CAGTGTTGCG      60
CTAGACTGGA AGGGCCCTCA AAAAATCATT GGAGTAGACA CTACTCCTCC AAAGAGCACC     120
AAGTTCCCCA AAAACTGGCA TGGAGTGAAC TTGAGATTCG ATGATGGGAC TTTAGGTGTG     180
GTTCAGTTCA TTAGGCCGTG CGTTTGGAGG GTTAGATACG ACCCTGGTTT CAAGACCTCT     240
GACGAGTATG GTGATGAGAA TACGAGGACA ATTGTGCAAG ATTATATGAG TACTCTGAGT     300
AATAAATTGG ATACTTATAG AGGTCTTACG TGGGAAACCA AGTGTGAGGA TTCGGGAGAT     360
TTCTTTACCT TCTCATCCAA GGTCACCGCC GTTGAAAAAT CCGAGCGGAC CCGCAACAAG     420
GTCGGCGATG GCCTCAGAAT TCACCTATGG AAAAGCCCTT TCCGCATCCA AGTAGTGCGC     480
ACCTTGACCC CTTTGAAGGA TCCTTACCCC ATTCCAAATG TAGCCGCAGC CGAAGCCCGT     540
GTGTCCGACA AGGTCGTTTG GCAAACGTCT CCCAAGACAT TCAGAAAGAA CCTGCATCCG     600
CAACACAAGA TGCTAAAGGA TACAGTTCTT GACATTGTCA AACCTGGACA TGGCGAGTAT     660
GTGGGGTGGG GAGAGATGGG AGGTATCCAG TTTATGAAGG AGCCAACATT CATGAACTAT     720
TTTAACTTCG ACAATATGCA ATACCAGCAA GTCTATGCCC AAGGTGCTCT CGATTCTCGC     780
GAGCCACTGT ACCACTCGGA TCCCTTCTAT CTTGATGTGA ACTCCAACCC GGAGCACAAG     840
AATATCACGG CAACCTTTAT CGATAACTAC TCTCAAATTG CCATCGACTT TGGAAAGACC     900
AACTCAGGCT ACATCAAGCT GGGAACCAGG TATGGTGGTA TCGATTGTTA CGGTATCAGT     960
GCGGATACGG TCCCGGAAAT TGTACGACTT TATACAGGTC TTGTTGGACG TTCAAAGTTG    1020
AAGCCCAGAT ATATTCTCGG GGCCCATCAA GCCTGTTATG GATACCAACA GGAAAGTGAC    1080
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GATGTCGATG TTCAGGACGG CTTCAGAACT TTCACCACCA ACCCACACAC TTTCCCTAAC    1200
CCCAAAGAGA TGTTTACTAA CTTGAGGAAT AATGGAATCA AGTGCTCCAC CAATATCACT    1260
CCTGTTATCA GCATTAACAA CAGAGAGGGT GGATACAGTA CCCTCCTTGA GGGAGTTGAC    1320
AAAAAATACT TTATCATGGA CGACAGATAT ACCGAGGGAA CAAGTGGGAA TGCGAAGGAT    1380
GTTCGGTACA TGTACTACGG TGGTGGTAAT AAGGTTGAGG TCGATCCTAA TGATGTTAAT    1440
GGTCGGCCAG ACTTTAAAGA CAACTATGAC TTCCCCGCGA ACTTCAACAG CAAACAATAC    1500
CCCTATCATG GTGGTGTGAG CTACGGTTAT GGGAACGGTA GTGCAGGTTT TTACCCGGAC    1560
CTCAACAGAA AGGAGGTTCG TATCTGGTGG GGAATGCAGT ACAAGTATCT CTTCGATATG    1620
GGACTGGAAT TTGTGTGGCA AGACATGACT ACCCCAGCAA TCCACACATC ATATGGAGAC    1680
ATGAAAGGGT TGCCCACCCG TCTACTCGTC ACCTCAGACT CCGTCACCAA TGCCTCTGAG    1740
AAAAAGCTCG CAATTGAAAC TTGGGCTCTC TACTCCTACA ATCTCCACAA AGCAACTTGG    1800
CATGGTCTTA GTCGTCTCGA ATCTCGTAAG AACAAACGAA ACTTCATCCT CGGGCGTGGA    1860
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GAATTCTGGA AGATATCGGT CTCTCAAGTT CTTTCTCTGG GCCTCAATGG TGTGTGCATC    1980
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GCGGGGTCTG ATACGGGTGG TTTTGAACCC TACCGTGATG CAAATGGGGT CGAGGAGAAA 2040
TACTGTAGCC CAGAGCTACT CATCAGGTGG TATACTGGTT CATTCCTCTT GCCGTGGCTC 2100
AGGAACCATT ATGTCAAAAA GGACAGGAAA TGGTTCCAGG AACCATACTC GTACCCCAAG 2160
CATCTTGAAA CCCATCCAGA ACTCGCAGAC CAAGCATGGC TCTATAAATC CGTTTTGGAG 2220
ATCTGTAGGT ACTATGTGGA GCTTAGATAC TCCCTCATCC AACTACTTTA CGACTGCATG 2280
TTTCAAAACG TAGTCGACGG TATGCCAATC ACCAGATCTA TGCTCTTGAC CGATACTGAG 2340
GATACCACCT TCTTCAACGA GAGCCAAAAG TTCCTCGACA ACCAATATAT GGCTGGTGAC 2400
GACATTCTTG TTGCACCCAT CCTCCACAGT CGCAAAGAAA TTCCAGGCGA AAACAGAGAT 2460
GTCTATCTCC CTCTTTACCA CACCTGGTAC CCCTCAAATT TGAGACCATG GGACGATCAA 2520
GGAGTCGCTT TGGGGAATCC TGTCGAAGGT GGTAGTGTCA TCAATTATAC TGCTAGGATT 2580
GTTGCACCCG AGGATTATAA TCTCTTCCAC AGCGTGGTAC CAGTCTACGT TAGAGAGGGT 2640
GCCATCATCC CGCAAATCGA AGTACGCCAA TGGACTGGCC AGGGGGGAGC CAACCGCATC 2700
AAGTTCAACA TCTACCCTGG AAAGGATAAG GAGTACTGTA CCTATCTTGA TGATGGTGTT 2760
AGCCGTGATA GTGCGCCGGA AGACCTCCCA CAGTACAAAG AGACCCACGA ACAGTCGAAG 2820
GTTGAAGGCG CGGAAATCGC AAAGCAGATT GGAAAGAAGA CGGGTTACAA CATCTCAGGA 2880
ACCGACCCAG AAGCAAAGGG TTATCACCGC AAAGTTGCTG TCACACAAAC GTCAAAAGAC 2940
AAGACGCGTA CTGTCACTAT TGAGCCAAAA CACAATGGAT ACGACCCTTC CAAAGAGGTG 3000
GGTGATTATT ATACCATCAT TCTTTGGTAC GCACCAGGTT TCGATGGCAG CATCGTCGAT 3060
GTGAGCAAGA CGACTGTGAA TGTTGAGGGT GGGGTGGAGC ACCAAGTTTA TAAGAACTCC 3120
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ATCACATGTA CTGCCGCTTA A 3201

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3213 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

ATGGCAGGAT TATCCGACCC TCTCAATTTC TGCAAAGCAG AGGACTACTA CGCTGCTGCC 60
AAAGGCTGGA GTGGCCCTCA GAAGATCATT CGCTATGACC AGACCCCTCC TCAGGGTACA 120
AAAGATCCGA AAAGCTGGCA TGCGGTAAAC CTTCCTTTCG ATGACGGGAC TATGTGTGTA 180
GTGCAATTCG TCAGACCCTG TGTTTGGAGG GTTAGATATG ACCCCAGTGT CAAGACTTCT 240
GATGAGTACG GCGATGAGAA TACGAGGACT ATTGTACAAG ACTACATGAC TACTCTGGTT 300
GGAAACTTGG ACATTTTCAG AGGTCTTACG TGGGTTTCTA CGTTGGAGGA TTCGGGCGAG 360
TACTACACCT TCAAGTCCGA AGTCACTGCC GTGGACGAAA CCGAACGGAC TCGAAACAAG 420
GTCGGCGACG GCCTCAAGAT TTACCTATGG AAAAATCCCT TTCGCATCCA GGTAGTGCGT 480
CTCTTGACCC CCCTGGTGGA CCCTTTCCCC ATTCCCAACG TAGCCAATGC CACAGCCCGT 540
GTGGCCGACA AGGTTGTTTG GCAGACGTCC CCGAAGACGT TCAGGAAAAA CTTGCATCCG 600
CAGCATAAGA TGTTGAAGGA TACAGTTCTT GATATTATCA AGCCGGGGCA CGGAGAGTAT 660
GTGGGTTGGG GAGAGATGGG AGGCATCGAG TTTATGAAGG AGCCAACATT CATGAATTAT 720
TTCAACTTTG ACAATATGCA ATATCAGCAG GTCTATGCAC AAGGCGCTCT TGATAGTCGT 780
GAGCCGTTGT ATCACTCTGA TCCCTTCTAT CTCGACGTGA ACTCCAACCC AGAGCACAAG 840
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AACTCAGGCT ACATCAAGCT GGGTACCAGG TATGGCGGTA TCGATTGTTA CGGTATCAGC 960
GCGGATACGG TCCCGGAGAT TGTGCGACTT TATACTGGAC TTGTTGGGCG TTCGAAGTTG 1020
AAGCCCAGGT ATATTCTCGG AGCCCACCAA GCTTGTTATG GATACCAGCA GGAAAGTGAC 1080
TTGCATGCTG TTGTTCAGCA GTACCGTGAC ACCAAGTTTC CGCTTGATGG GTTGCATGTC 1140
GATGTCGACT TTCAGGACAA TTTCAGAACG TTTACCACTA ACCCGATTAC GTTCCCTAAT 1200
CCCAAAGAAA TGTTTACCAA TCTAAGGAAC AATGGAATCA AGTGTTCCAC CAACATCACC 1260
CCTGTTATCA GTATCAGAGA TCGCCCGAAT GGGTACAGTA CCCTCAATGA GGGATATGAT 1320
AAAAAGTACT TCATCATGGA TGACAGATAT ACCGAGGGGA CAAGTGGGGA CCCGCAAAAT 1380
GTTCGATACT CTTTTTACGG CGGTGGGAAC CCGGTTGAGG TTAACCCTAA TGATGTTTGG 1440
GCTCGGCCAG ACTTTGGAGA CAATTATGAC TTCCCTACGA ACTTCAACTG CAAAGACTAC 1500
CCCTATCATG GTGGTGTGAG TTACGGATAT GGGAATGGCA CTCCAGGTTA CTACCCTGAC 1560
CTTAACAGAG AGGAGGTTCG TATCTGGTGG GGATTGCAGT ACGAGTATCT CTTCAATATG 1620
GGACTAGAGT TTGTATGGCA AGATATGACA ACCCCAGCGA TCCATTCATC ATATGGAGAC 1680
ATGAAAGGGT TGCCCACCCG TCTGCTCGTC ACCGCCGACT CAGTTACCAA TGCCTCTGAG 1740
AAAAAGCTCG CAATTGAAAG TTGGGCTCTT TACTCCTACA ACCTCCATAA AGCAACCTTC 1800
CACGGTCTTG GTCGTCTTGA GTCTCGTAAG AACAAACGTA ACTTCATCCT CGGACGTGGT 1860
AGTTACGCCG GTGCCTATCG TTTTGCTGGT CTCTGGACTG GAGATAACGC AAGTACGTGG 1920
GAATTCTGGA AGATTTCGGT CTCCCAAGTT CTTTCTCTAG GTCTCAATGG TGTGTGTATA 1980

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GCGGGGTCTG ATACGGGTGG TTTTGAGCCC GCACGTACTG AGATTGGGGA GGAGAAATAT    2040
TGCAGTCCGG AGCTACTCAT CAGGTGGTAT ACTGGATCAT TCCTTTTGCC ATGGCTTAGA    2100
AACCACTACG TCAAGAAGGA CAGGAAATGG TTCCAGGAAC CATACGCGTA CCCCAAGCAT    2160
CTTGAAACCC ATCCAGAGCT CGCAGATCAA GCATGGCTTT ACAAATCTGT TCTAGAAATT    2220
TGCAGATACT GGGTAGAGCT AAGATATTCC CTCATCCAGC TCCTTTACGA CTGCATGTTC    2280
CAAAACGTGG TCGATGGTAT GCCACTTGCC AGATCTATGC TCTTGACCGA TACTGAGGAT    2340
ACGACCTTCT TCAATGAGAG CCAAAAGTTC CTCGATAACC AATATATGGC TGGTGACGAC    2400
ATCCTTGTAG CACCCATCCT CCACAGCCGT AACGAGGTTC CGGGAGAGAA CAGAGATGTC    2460
TATCTCCCTC TATTCCACAC CTGGTACCCC TCAAACTTGA GACCGTGGGA CGATCAGGGA    2520
GTCGCTTTAG GGAATCCTGT CGAAGGTGGC AGCGTTATCA ACTACACTGC CAGGATTGTT    2580
GCCCCAGAGG ATTATAATCT CTTCCACAAC GTGGTGCCGG TCTACATCAG AGAGGGTGCC    2640
ATCATTCCGC AAATTCAGGT ACGCCAGTGG ATTGGCGAAG GAGGGCCTAA TCCCATCAAG    2700
TTCAATATCT ACCCTGGAAA GGACAAGGAG TATGTGACGT ACCTTGATGA TGGTGTTAGC    2760
CGCGATAGTG CACCAGATGA CCTCCCGCAG TACCGCGAGG CCTATGAGCA AGCGAAGGTC    2820
GAAGGCAAAG ACGTCCAGAA GCAACTTGCG GTCATTCAAG GGAATAAGAC TAATGACTTC    2880
TCCGCCTCCG GGATTGATAA GGAGGCAAAG GGTTATCACC GCAAAGTTTC TATCAAACAG    2940
GAGTCAAAAG ACAAGACCCG TACTGTCACC ATTGAGCCAA AACACAACGG ATACGACCCC    3000
TCTAAGGAAG TTGGTAATTA TTATACCATC ATTCTTTGGT ACGCACCGGG CTTTGACGGC    3060
AGCATCGTCG ATGTGAGCCA GGCGACCGTG AACATCGAGG GCGGGGTGGA ATGCGAAATT    3120
TTCAAGAACA CCGGCTTGCA TACGGTTGTA GTCAACGTGA AAGAGGTGAT CGGTACCACA    3180
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SEQ. ID. NO. 11

SEQUENCE TYPE: NUCLEIC ACID
MOLECULE TYPE: DNA (GENOMIC)
ORIGINAL SOURCE: ALGAL
SEQUENCE LENGTH: 3279 BP
STRANDEDNESS: DOUBLE
SEQUENCE:

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    61 GCGGATATCC GATCGGGCAT TCGCATTCAA TCCGCTCTTC CGGCCGTGCG CAACGCTGTG
   121 CGCAGGAGCA AACATTACAA TGTATCCATG ACCGCATTGT CTGACAAGCA AACCGCTATC
   181 AGTATTGGCC CTGACAATCC GGACGGTATC AACTACCAAA ACTACGATTA CATCCCTGTA
   241 GCGGGCTTTA CGCCCCTCTC CAACACCAAC TGGTATGCTG CCGGCTCTTC CACTCCGGGC
   301 GGCATCACCG ACTGGACCGC TACCATGAAT GTCAAATTCG ACCGCATTGA CAATCCGTCG
   361 TACTCCAATA ACCATCCTGT TCAGATTCAG GTCACGTCGT ACAACAACAA CAGCTTCAGG
   421 ATTCGCTTCA ACCCTGATGG CCCCATTCGT GACGTCTCTC GAGGACCTAT CCTGAAACAG
   481 CAACTCACTT GGATTCGAAA CCAGGAGCTG GCGCAGGGAT GTAATCCGAA CATGAGCTTC
   541 TCTCCTGAAG GTTTTTTGTC TTTTGAAACC AAAGACCTAA ACGTTATAAT CTACGGCAAC
   601 TGCAAGATGA GAGTCACGAA GAAGGATGGC TACCTCGTCA TGGAGAATGA CGAGTGCAAC
   661 TCGCAATCAG ATGGCAATAA GTGTAGAGGA TTGATGTACG TTGACCGGCT ATACGGTAAT
   721 GCTATTGCTT CCGTACAAAC GAATTTTCAC AAAGACACTT CTCGGAACGA GAAATTCTAT
   781 GGTGCAGGTG AAGTCAACTG TCGCTATGAG GAGCAGGGTA AGGCGCCGAC TTATGTTCTA
   841 GAACGCTCTG GACTCGCCAT GACCAATTAC AATTACGACA ACTTGAACTA CAACCAACCA
   901 GACGTCGTTC CTCCAGGTTA TCCCGACCAT CCCAACTACT ACATTCCAAT GTACTACGCA
   961 GCACCGTGGT TGGTCGTTCA GGGATGCGCG GGGACATCGA AGCAATACTC GTACGGTTGG
  1021 TTTATGGACA ATGTCTCTCA GTCGTACATG AACACTGGAG ATACGGCGTG GAACTGCGGA
  1081 CAGGAAAACC TGGCATACAT GGGCGCGCAA TACGGGCCAT TTGATCAGCA CTTTGTGTAT
  1141 GGTGATGGAG ATGGCCTTGA AGATGTCGTC AAAGCGTTCT CCTTTCTTCA AGGAAAGGAG
  1201 TTCGAAGACA AAAAACTCAA CAAGCGTTCT GTAATGCCTC CGAAGTACGT GTTTGGTTTC
  1261 TTCCAGGGTG TTTTCGGTGC ACTTTCACTG TTGAAGCAGA ATCTGCCTGC CGGAGAGAAC
  1321 AACATCTCAG TGCAAGAGAT TGTGGAGGGT TACCAGGATA ACGACTACCC CTTTGAAGGG
  1381 CTCGCGGTAG ATGTTGATAT GCAAGATGAT CTGCGAGTGT TTACTACCAA ACCAGAATAT
  1441 TGGTCGGCAA ACATGGTAGG CGAAGGCGGT GATCCTAATA ACAGATCAGT CTTTGAATGG
  1501 GCACATGACA GGGGCCTTGT CTGTCAGACG AACGTAACTT GCTTCTTGAG GAACGATAAC
  1561 AGTGGGAAAC CATACGAAGT GAATCAGACA TTGAGGGAGA AACAGTTGTA TACGAAGAAT
  1621 GATTCCTTGA ACAACACCGA TTTTGGAACT ACCTCGGATG GGCCTGGCGA TGCGTACATT
  1681 GGACATTTGG ACTATGGTGG TGGAGTGGAG TGTGATGCAA TCTTCCCAGA CTGGGGTCGA
  1741 CCAGACGTGG CTCAATGGTG GGGAGAAAAC TACAAGAAGC TGTTCAGCAT TGGTCTCGAT
  1801 TTCGTGTGGC AGGATATGAC GGTACCTGCG ATGATGCCGC ACCGACTCGG TGATGCTGTC
  1861 AACAAAAATT CCGGTAGTTC GGCGCCGGGC TGGCCGAATG AGAACGATCC ATCCAACGGA
  1921 CGATACAACT GGAAATCTTA TCATCCGCAA GTGCTCGTGA CCGACATGCG CTATGGTGCA
  1981 GAGTATGGAA GGGAACCGAT GGTGTCTCAA CGCAACATTC ACGCCTACAC TCTTTGTGAA
  2041 TCTACCAGAC GGGAGGGAAT TGTGGGAAAC GCAGACAGTT TGACCAAGTT CCGCCGCAGT
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2101 TACATCATCA GTCGAGGAGG TTACATCGGT AACCAGCATT TCGGAGGGAT GTGGGTTGGG
2161 GACAACAGTG CCACAGAATC CTACCTCCAA ATGATGTTGG CGAACATTAT CAACATGAAT
2221 ATGTCGTGCC TCCCGCTAGT TGGCTCTGAT ATTGGCGGGT TCACCCAGTA CAATGATGCG
2281 GGCGACCCAA CCCCCGAGGA TTTGATGGTA AGATTCGTGC AGGCTGGCTG TCTGCTACCG
2341 TGGTTCAGAA ACCACTATGA CAGGTGGATT GAGTCCAAGA AGCACGGGAA GAAATACCAG
2401 GAGTTATACA TGTACCCGGG GCAAAAGGAT ACGTTGAAGA AGTTCGTTGA ATTCCGCTAC
2461 CGCTGGCAGG AGGTTTTGTA CACAGCCATG TACCAAAATG CTACCACTGG AGAGCCGATC
2521 ATCAAGGCGG CGCCCATGTA CAACAACGAC GTCAACGTGT ATAAATCGCA GAATGATCAT
2581 TTCCTTCTCG GTGGACATGA CGGCTATCGT ATTCTCTGCG CACCTGTTGT GCGCGAAAAT
2641 GCGACAAGTC GCGAAGTGTA CCTGCCTGTG TATAGCAAGT GGTTCAAATT CGGACCGGAC
2701 TTTGACACTA AGCCCTTGGA AAATGAGATT CAAGGAGGTC AGACGCTTTA TAATTACGCT
2761 GCACCGCTGA ACGATTCGCC GATATTTGTG AGGGAAGGGA CTATTCTTCC GACACGGTAC
2821 ACGCTGGACG GTGTGAACAA ATCTATCAAC ACGTACACAG ACAATGATCC GCTTGTATTT
2881 GAGCTGTTCC CTCTCGAAAA CAACCAGGCG CATGGCTTGT TCTATCATGA TGATGGCGGT
2941 GTCACCACCA ACGCTGAAGA CTTTGGCAAG TATTCTGTGA TCAGTGTGAA GGCCGCGCAG
3001 GAAGGTTCTC AAATGAGTGT CAAGTTTGAC AATGAAGTTT ATGAACACCA ATGGGGAGCA
3061 TCGTTCTATG TTCGTGTTCG TAATATGGGT GCTCCGTCTA ACATCAACGT ATCTTCTCAG
3121 ATTGGTCAAC AGGACATGCA ACAGAGCTCC GTGAGTTCCA GGGCGCAAAT GTTCACTAGT
3181 GCTAACGATG GCGAGTACTG GGTTGACCAG AGCACGAACT CGTTGTGGCT CAAGTTGCCT
3241 GGTGCAGTTA TCCAAGACGC TGCGATCACT GTTCGTTGA
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SEQ. ID. NO. 12

SEQUENCE TYPE: NUCLEIC ACID
MOLECULE TYPE: DNA (GENOMIC)
ORIGINAL SOURCE: ALGAL
SEQUENCE LENGTH: 1712 BP
STRANDEDNESS: DOUBLE
SEQUENCE:

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  61 CATGATTCAG ATCCTGACTA CTATATTCCG ATGTACTTTG CGGCACCATG GGTGATCGCA
 121 CATGGATATC GTGGCACCAG CGACCAGTAC TCTTATGGAT GGTTTTTGGA CAATGTATCC
 181 CAGTCCTACA CAAACACTGG CGATGATGCA TGGGCTGGTC AGAAGGATTT GGCGTACATG
 241 GGGGCACAAT GTGGGCCTTT CGATCAACAT TTTGTGTATG AGGCTGGAGA TGGACTTGAA
 301 GACGTTGTGA CCGCATTCTC TTATTTGCAA GGCAAGGAAT ATGAGAACCA GGGACTGAAT
 361 ATACGTTCTG CAATGCCTCC GAAGTACGTT TTCGGATTTT TCCAAGGCGT ATTCGGAGCC
 421 ACATCGCTGC TAAGGGACAA CTTACCTGCC GGCGAGAACA ACGTCTCTTT GGAAGAAATT
 481 GTTGAAGGAT ATCAAAATCA GAACGTGCCA TTTGAAGGTC TTGCTGTGGA TGTTGATATG
 541 CAAGATGACT TGAGAGTGTT CACTACGAGA CCAGCGTTTT GGACGGCAAA CAAGGTGGGG
 601 GAAGGCGGTG ATCCAAACAA CAAGTCAGTG TTTGAGTGGG CACATGACAG GGGCCTTGTC
 661 TGCCAGACGA ATGTAACTTG CTTCTTGAAG AACGAGAAAA ATCCTTACGA AGTGAATCAG
 721 TCATTGAGGG AGAAGCAGTT GTATACGAAG AGTGATTCCT TGGACAACAT TGATTTTGGA
 781 ACTACTCCAG ATGGGCCTAG CGATGCGTAC ATTGGACACT TAGACTACGG TGGTGGTGTG
 841 GAGTGTGATG CACTATTCCC AGACTGGGGT CGACCAGACG TGGCTCAATG GTGGGGCGAT
 901 AACTACAAGA AACTATTCAG CATTGGTCTC GATTTCGTCT GGCAAGATAT GACGGTACCT
 961 GCGATGATGC CGCACCGACT CGGTGACCCT GTCGGCACAA ATTCCGGTGA GACGGCGCCG
1021 GGCTGGCCGA ATGATAAGGA TCCATCCAAC GGACGATACA ATTGGAAGTC TTACCATCCG
1081 CAAGTGCTCG TGACTGACAT GAGGTATGAC GATTACGGAA GAGATCCCAT TGTTACGCAA
1141 CGCAATCTCC ATGCCTACAC TCTTTGTGAG TCTACTAGGA GGGAAGGCAT TGTTGGAAAC
1201 GCAGATAGTC TGACGAAGTT CCGCCGCAGC TATATTATCA GTCGTGGAGG CTACATCGGT
1261 AATCAGCACT TTGGTGGGAT GTGGGTAGGA GACAACTCTT CTACGGAAGA CTACCTCGCA
1321 ATGATGGTTA TCAACGTTAT CAACATGAAC ATGTCCGGTG TCCCGCTCGT TGGTTCCGAT
1381 ATTGGAGGTT TCACGGAGCA TGACAAGAGA AACCCTTGCA CACCGGACTT GATGATGAGA
1441 TTTGTGCAGG CTGGATGCTT GCTACCGTGG TTCAGGAACC ACTACGATAG GTGGATCGAG
1501 AGCAAGAAAC ACGGAAAGAA CTACCAAGAG TTGTACATGT ACCGCGACCA CTTGGACGCC
1561 TTGAGAAGTT TTGTGGAACT CCGCTATCGC TGGCAGGAAG TGTTATACAC AGCCATGTAT
1621 CAGAATGCTT TGAACGGGAA GCCGATCATC AAAACGGTCT CCATGTACAA CAACGATATG
1681 AACGTCAAAG ATGCTCAGAA TGACCACTTC CT
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CLAIMS

1. A process of preparing a medium that comprises an anti-oxidant and at least one other component, the process comprising preparing *in situ* in the medium the anti-oxidant; and wherein the anti-oxidant is prepared from a glucan by use of recombinant DNA techniques.

2. A process according to claim 1, wherein the glucan comprises α-1,4 links.

3. A process according to claim 1 or claim 2 wherein the glucan is starch.

4. A process according to any one of the preceding claims wherein the glucan is a substrate for a recombinant enzyme such that contact of the glucan with the recombinant enzyme yields the anti-oxidant.

5. A process according to any one of claims 1 to 4, wherein the enzyme is a glucan lyase.

6. A process according to claim 5, wherein the enzyme is an α-1,4-glucan lyase.

7. A process according to claim 6, wherein the enzyme comprises any one of the sequences shown as SEQ ID Nos 1-6, or a variant, homologue or fragment thereof.

8. A process according to claim 7, wherein the enzyme is any one of the sequences shown as SEQ ID Nos 1-6.

9. A process according to any one of claims 5 to 8, wherein the enzyme is encoded by a nucleotide sequence comprising any one of the sequences shown as SEQ ID Nos 7-12, or a variant, homologue or fragment thereof.

10. A process according to claim 9, wherein the enzyme is encoded by a nucleotide sequence having any one of the sequences shown as SEQ ID Nos 7-12.

11. A process according to any one of the preceding claims, wherein the anti-oxidant is anhydrofructose.

12. A process according to claim 11, wherein the anti-oxidant is 1,5-D-anhydrofructose.

13. A process according to any one of the preceding claims, wherein the medium is, or is used in the preparation of, a foodstuff.

14. A process according to claim 13, wherein the foodstuff is a beverage.

15. A process according to claim 14, wherein the beverage is an alcoholic beverage.

16. A process according to claim 14, wherein the beverage is a wine.

17. A process according to any one of the preceding claims, wherein the anti-oxidant is prepared *in situ* in the component and is then released into the medium.

18. A process according to any one of the preceding claims, wherein the component is a plant or a part thereof.

19. A process according to claim 18, wherein the component is all or part of a cereal or a fruit.

20. A process according to claim 20, wherein the component is all or part of a grape.

21. A process of preparing a medium that comprises an anti-oxidant and at least one other component, the process comprising preparing *in situ* in the medium the anti-oxidant; and wherein the anti-oxidant is prepared by use of a recombinant glucan lyase.

22. A process according to claim 21 wherein the glucan lyase is that as defined in any one of claims 6 to 10.

23. A medium prepared by the process according to any one of the preceding claims.

24. Use of anhydrofructose as an anti-oxidant for a medium comprising at least one other component, wherein the anhydrofructose is prepared *in situ* in the medium.

25. Use of anhydrofructose as a means for imparting or improving stress tolerance in a plant, wherein the anhydrofructose is prepared *in situ* in the plant.

26. Use of anhydrofructose as a means for imparting or improving the transformation of a grape, wherein the anhydrofructose is prepared *in situ* in the grape.

27. Use of glucan lyase as a means for imparting or improving stress tolerance in a plant, wherein the glucan lyase is prepared *in situ* in the plant.

28. Use of glucan lyase as a means for imparting or improving the transformation of a grape, wherein the glucan lyase is prepared *in situ* in the grape.

29. Use of a nucleotide sequence coding for a glucan lyase as a means for imparting or improving stress tolerance in a plant, wherein the nucleotide sequence is expressed *in situ* in the plant.

30. Use of a nucleotide sequence coding for a glucan lyase as a means for imparting or improving the transformation of a grape, wherein the nucleotide sequence is expressed *in situ* in the grape.

31. A process or medium substantially as described herein.

ABSTRACT

A PROCESS OF PREPARING AN ANTI-OXIDANT

A process of preparing an anti-oxidant is described. The process comprises preparing a medium that comprises an anti-oxidant and at least one other component. The process comprises preparing *in situ* in the medium the anti-oxidant. The anti-oxidant is prepared from either a glucan by use of recombinant DNA techniques and/or by use of a recombinant glucan lyase.